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(54) Title: LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS

(57) Abstract

Engineered fluorescent proteins, nucleic acids encoding them and methods of use.

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LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS

BACKGROUND OF THE INVENTION

This application claims the benefit of the earlier filing date of a United States provisional patent application serial number 60/024,050 filed on August 16, 1996 entitled "Long Wavelength Mutant Fluorescent Proteins" and patent application serial number 08/706,408 filed on August 30, 1996 entitled "Long Wavelength Engineered Fluorescent Proteins," both of which are herein incorporated by reference.

This invention was made in part with Government support under grant no. MCB 9418479 awarded by the National Science Foundation. The Government may have rights in this invention.

Fluorescent molecules are attractive as reporter molecules in many assay systems because of their high sensitivity and ease of quantification. Recently, fluorescent proteins have been the focus of much attention because they can be produced *in vivo* by biological systems, and can be used to trace intracellular events without the need to be introduced into the cell through microinjection or permeabilization. The green fluorescent protein of *Aequorea victoria* is particularly interesting as a fluorescent protein. A cDNA for the protein has been cloned. (D.C. Prasher et al., "Primary structure of the *Aequorea victoria* green-fluorescent protein," *Gene* (1992) 111:229-33.) Not only can the primary amino acid sequence of the protein be expressed from the cDNA, but the expressed protein can fluoresce. This indicates that the protein can undergo the cyclization and oxidation believed to be necessary for fluorescence. *Aequorea* green fluorescent protein ("GFP") is a stable, proteolysis-resistant single chain of 238 residues and has two absorption maxima at around 395 and 475 nm. The relative amplitudes of these two peaks is sensitive to environmental factors (W. W. Ward. *Bioluminescence and Chemiluminescence* (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman *Biochemistry* 21:4535-4540 (1982); W. W. Ward et al. *Photochem. Photobiol.* 35:803-808 (1982)) and illumination history (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995)), presumably reflecting two or more ground states. Excitation at the primary absorption peak of 395 nm yields an emission maximum at 508 nm with a quantum yield of 0.72-0.85 (O. Shimomura and F.H. Johnson *J. Cell. Comp. Physiol.* 59:223 (1962);

J. G. Morin and J. W. Hastings, *J. Cell. Physiol.* 77:313 (1971); H. Morise et al. *Biochemistry* 13:2656 (1974); W. W. Ward *Photochem. Photobiol. Reviews* (Smith, K. C. ed.) 4:1 (1979); A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995); D. C. Prasher *Trends Genet.* 11:320-323 (1995); M. Chalfie *Photochem. Photobiol.* 62:651-656 (1995); W. W. Ward. *Bioluminescence and Chemiluminescence* (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman *Biochemistry* 21:4535-4540 (1982); W. W. Ward et al. *Photochem. Photobiol.* 35:803-808 (1982)). The fluorophore results from the autocatalytic cyclization of the polypeptide backbone between residues Ser^{65} and Gly^{67} and oxidation of the □-ß bond of Tyr^{66} (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995); C. W. Cody et al. *Biochemistry* 32:1212-1218 (1993); R. Heim et al. *Proc. Natl. Acad. Sci.* USA 91:12501-12504 (1994)). Mutation of Ser^{65} to Thr (S65T) simplifies the excitation spectrum to a single peak at 488 nm of enhanced amplitude (R. Heim et al. *Nature* 373:664-665 (1995)), which no longer gives signs of conformational isomers (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995)).

Fluorescent proteins have been used as markers of gene expression, tracers of cell lineage and as fusion tags to monitor protein localization within living cells. (M. Chalfie et al., "Green fluorescent protein as a marker for gene expression," *Science* 263:802-805; A.B. Cubitt et al., "Understanding, improving and using green fluorescent proteins," *TIBS 20*, November 1995, pp. 448-455. U.S. patent 5,491,084, M. Chalfie and D. Prasher. Furthermore, engineered versions of *Aequorea* green fluorescent protein have been identified that exhibit altered fluorescence characteristics, including altered excitation and emission maxima, as well as excitation and emission spectra of different shapes. (R. Heim et al., "Wavelength mutations and posttranslational autoxidation of green fluorescent protein," *Proc. Natl. Acad. Sci. USA*, (1994) 91:12501-04; R. Heim et al., "Improved green fluorescence," *Nature* (1995) 373:663-665.) These properties add variety and utility to the arsenal of biologically based fluorescent indicators.

There is a need for engineered fluorescent proteins with varied fluorescent properties.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1B. (A) Schematic drawing of the backbone of GFP produced by

Molscript (J.P. Kraulis, *J. Appl. Cryst.*, 24:946 (1991)). The chromophore is shown as a ball and stick model. (B) Schematic drawing of the overall fold of GFP. Approximate residue numbers mark the beginning and ending of the secondary structure elements.

Figs. 2A-2C. (A) Stereo drawing of the chromophore and residues in the immediate vicinity. Carbon atoms are drawn as open circles, oxygen is filled and nitrogen is shaded. Solvent molecules are shown as isolated filled circles. (B) Portion of the final $2F_o$-F_c electron density map contoured at 1.0 □, showing the electron density surrounding the chromophore. (C) Schematic diagram showing the first and second spheres of coordination of the chromophore. Hydrogen bonds are shown as dashed lines and have the indicated lengths in Å. Inset: proposed structure of the carbinolamine intermediate that is presumably formed during generation of the chromophore.

Fig. 3 depicts the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of an *Aequorea* green fluorescent protein.

Fig. 4 depicts the nucleotide sequence (SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) of the engineered *Aequorea*-related fluorescent protein S65G/S72A/T203Y utilizing preferred mammalian codons and optimal Kozak sequence.

Figs. 5-1 to 5-28 present the coordinates for the crystal structure of *Aequorea*-related green fluorescent protein S65T.

Fig. 6 shows the fluorescence excitation and emission spectra for engineered fluorescent proteins 20A and 10C (Table F). The vertical line at 528 nm compares the emission maxima of 10C, to the left of the line, and 20A, to the right of the line.

SUMMARY OF THE INVENTION

This invention provides functional engineered fluorescent proteins with varied fluorescence characteristics that can be easily distinguished from currently existing green and blue fluorescent proteins. Such engineered fluorescent proteins enable the simultaneous measurement of two or more processes within cells and can be used as fluorescence energy donors or acceptors when used to monitor protein-protein interactions through FRET. Longer wavelength engineered fluorescent proteins are particularly useful because photodynamic toxicity and auto-fluorescence of cells are significantly reduced at longer wavelengths. In particular, the introduction of the substitution T203X, wherein X is an aromatic amino acid, results in an increase in the excitation and emission wavelength

maxima of *Aequorea*-related fluorescent proteins.

In one aspect, this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 nm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

In one aspect this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at T203 and, in particular, T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein. In one embodiment, the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I. In another embodiment, the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S65G/V68L/Q69K/S72A/T203Y; S72A/S65G/V68L/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W. In another embodiment, the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W. In another embodiment, the amino acid sequence further comprises a mutation from Table A. In another embodiment, the amino acid sequence further comprises a folding mutation. In another embodiment, the nucleotide sequence encoding the protein differs from the nucleotide sequence of SEQ ID NO:1 by the substitution of at least one codon by a preferred mammalian codon. In another embodiment, the nucleic acid molecule encodes a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

In another aspect, this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green

fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein. In one embodiment, amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,
V61X, wherein X is selected from F, Y, H and C,
T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,
V68X, wherein X is selected from F, Y and H,
Q69X, wherein X is selected from K, R, E and G,
Q94X, wherein X is selected from D, E, H, K and N,
N121X, wherein X is selected from F, H, W and Y,
Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,
H148X, wherein X is selected from F, Y, N, K, Q and R,
V150X, wherein X is selected from F, Y and H,
F165X, wherein X is selected from H, Q, W and Y,
I167X, wherein X is selected from F, Y and H,
Q183X, wherein X is selected from H, Y, E and K,
N185X, wherein X is selected from D, E, H, K and Q,
L220X, wherein X is selected from H, N, Q and T,
E222X, wherein X is selected from N and Q, or
V224X, wherein X is selected from H, N, Q, T, F, W and Y.

In a further aspect, this invention provides an expression vector comprising expression control sequences operatively linked to any of the aforementioned nucleic acid molecules. In a further aspect, this invention provides a recombinant host cell comprising the aforementioned expression vector.

In another aspect, this invention provides a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 nm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the

electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

In another aspect, this invention provides a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution at T203, and in particular, T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein. In one embodiment, the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I. In another embodiment, the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W. In another embodiment, the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W. In another embodiment, the amino acid sequence further comprises a folding mutation. In another embodiment, the engineered fluorescent protein is part of a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

In another aspect this invention provides a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222, or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

In another aspect, this invention provides a fluorescently labelled antibody comprising an antibody coupled to any of the aforementioned functional engineered fluorescent proteins. In one embodiment, the fluorescently labelled antibody is a fusion protein wherein the fusion protein comprises the antibody fused to the functional engineered fluorescent protein.

In another aspect, this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding an antibody fused to a nucleotide sequence encoding a

functional engineered fluorescent protein of this invention.

In another aspect, this invention provides a fluorescently labelled nucleic acid probe comprising a nucleic acid probe coupled to a functional engineered fluorescent protein whose amino acid sequence of this invention. The fusion can be through a linker peptide.

In another aspect, this invention provides a method for determining whether a mixture contains a target comprising contacting the mixture with a fluorescently labelled probe comprising a probe and a functional engineered fluorescent protein of this invention; and determining whether the target has bound to the probe. In one embodiment, the target molecule is captured on a solid matrix.

In another aspect, this invention provides a method for engineering a functional engineered fluorescent protein having a fluorescent property different than *Aequorea* green fluorescent protein, comprising substituting an amino acid that is located no more than 0.5 nm from any atom in the chromophore of an *Aequorea*-related green fluorescent protein with another amino acid; whereby the substitution alters a fluorescent property of the protein. In one embodiment, the amino acid substitution alters the electronic environment of the chromophore.

In another aspect, this invention provides a method for engineering a functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein comprising substituting amino acids in a loop domain of an *Aequorea*-related green fluorescent protein with amino acids so as to create a consensus sequence for phosphorylation or for proteolysis.

In another aspect, this invention provides a method for producing fluorescence resonance energy transfer comprising providing a donor molecule comprising a functional engineered fluorescent protein this invention; providing an appropriate acceptor molecule for the fluorescent protein; and bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy transfer.

In another aspect, this invention provides a method for producing fluorescence resonance energy transfer comprising providing an acceptor molecule comprising a functional engineered fluorescent protein of this invention; providing an appropriate donor molecule for the fluorescent protein; and bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy

transfer. In one embodiment, the donor molecule is a engineered fluorescent protein whose amino acid sequence comprises the substitution T203I and the acceptor molecule is an engineered fluorescent protein whose amino acid sequence comprises the substitution T203X. wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

In another aspect, this invention provides a crystal of a protein comprising a fluorescent protein with an amino acid sequence substantially identical to SEQ ID NO: 2, wherein said crystal diffracts with at least a 2.0 to 3.0 angstrom resolution.

In another embodiment, this invention provides computational method of designing a fluoresent protein comprising determining from a three dimensional model of a crystallized fluorescent protein comprising a fluorescent protein with a bound ligand, at least one interacting amino acid of the fluorescent protein that interacts with at least one first chemical moiety of the ligand, and selecting at least one chemical modification of the first chemical moiety to produce a second chemical moiety with a structure to either decrease or increase an interaction between the interacting amino acid and the second chemical moiety compared to the interaction between the interacting amino acid and the first chemical moiety.

In another embodiment, this invention provides a computational method of modeling the three dimensional structure of a fluorescent protein comprising determining a three dimensional relationship between at least two atoms listed in the atomic coordinates of Figs. 5-1 to 5-28.

In another embodiment, this invention provides a device comprising a storage device and, stored in the device, at least 10 atomic coordinates selected from the atomic coordinates listed in Figs. 5-1 to 5-28. In one embodiment, the storage device is a computer readable device that stores code that receives as input the atomic coordinates. In another embodiment, the computer readable device is a floppy disk or a hard drive.

DETAILED DESCRIPTION OF THE INVENTION

I. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which

this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are defined below.

"Binding pair" refers to two moieties (e.g. chemical or biochemical) that have an affinity for one another. Examples of binding pairs include antigen/antibodies, lectin/avidin, target polynucleotide/probe oligonucleotide, antibody/anti-antibody, receptor/ligand, enzyme/ligand and the like. "One member of a binding pair" refers to one moiety of the pair, such as an antigen or ligand.

"Nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and, unless otherwise limited, encompasses known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides. It will be understood that when a nucleic acid molecule is represented by a DNA sequence, this also includes RNA molecules having the corresponding RNA sequence in which "U" replaces "T."

"Recombinant nucleic acid molecule" refers to a nucleic acid molecule which is not naturally occurring, and which comprises two nucleotide sequences which are not naturally joined together. Recombinant nucieic acid molecules are produced by artificial recombination, e.g., genetic engineering techniques or chemical synthesis.

Reference to a nucleotide sequence "encoding" a polypeptide means that the sequence, upon transcription and translation of mRNA, produces the polypeptide. This includes both the coding strand, whose nucleotide sequence is identical to mRNA and whose sequence is usually provided in the sequence listing, as well as its complementary strand, which is used as the template for transcription. As any person skilled in the art recognizes, this also includes all degenerate nucleotide sequences encoding the same amino acid sequence. Nucleotide sequences encoding a polypeptide include sequences containing introns.

"Expression control sequences" refers to nucleotide sequences that regulate the expression of a nucleotide sequence to which they are operatively linked. Expression control sequences are "operatively linked" to a nucleotide sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleotide sequence. Thus, expression control sequences can include appropriate

promoters, enhancers, transcription terminators, a start codon (i.e., ATG) in front of a protein-encoding gene, splicing signals for introns, maintenance of the correct reading frame of that gene to permit proper translation of the mRNA, and stop codons.

"Naturally-occurring" as used herein, as applied to an object, refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences, such as when the appropriate molecules (e.g., inducers and polymerases) are bound to the control or regulatory sequence(s).

"Control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding and non-coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

"Isolated polynucleotide" refers a polynucleotide of genomic, cDNA, or synthetic origin or some combination there of, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with the cell in which the "isolated polynucleotide" is found in nature, or (2) is operably linked to a polynucleotide which it is not linked to in nature.

"Polynucleotide" refers to a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

The term "probe" refers to a substance that specifically binds to another substance (a "target"). Probes include, for example, antibodies, nucleic acids, receptors and

their ligands.

"Modulation" refers to the capacity to either enhance or inhibit a functional property of biological activity or process (e.g., enzyme activity or receptor binding); such enhancement or inhibition may be contingent on the occurrence of a specific event, such as activation of a signal transduction pathway, and/or may be manifest only in particular cell types.

The term "modulator" refers to a chemical (naturally occurring or non-naturally occurring), such as a synthetic molecule (e.g., nucleic acid, protein, non-peptide, or organic molecule), or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues. Modulators can be evaluated for potential activity as inhibitors or activators (directly or indirectly) of a biological process or processes (e.g., agonist, partial antagonist, partial agonist, inverse agonist, antagonist, antineoplastic agents, cytotoxic agents, inhibitors of neoplastic transformation or cell proliferation, cell proliferation-promoting agents, and the like) by inclusion in screening assays described herein. The activity of a modulator may be known, unknown or partially known.

The term "test chemical" refers to a chemical to be tested by one or more screening method(s)of the invention as a putative modulator. A test chemical is usually not known to bind to the target of interest. The term "control test chemical" refers to a chemical known to bind to the target (e.g., a known agonist, antagonist, partial agonist or inverse agonist). Usually, various predetermined concentrations of test chemicals are used for screening, such as .01 μM, .1 μM, 1.0 μM, and 10.0 μM.

The term "target" refers to a biochemical entity involved a biological process. Targets are typically proteins that play a useful role in the physiology or biology of an organism. A therapeutic chemical binds to target to alter or modulate its function. As used herein targets can include cell surface receptors, G-proteins, kinases, ion channels, phopholipases and other proteins mentioned herein.

The term "label" refers to a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, fluorescent dyes, fluorescent proteins, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, dioxigenin, or haptens and proteins for which antisera or monoclonal antibodies are available. For example, polypeptides of this invention can be made as detectible labels, by e.g., incorporating a them as into a polypeptide, and

used to label antibodies specifically reactive with the polypeptide. A label often generates a measurable signal, such as radioactivity, fluorescent light or enzyme activity, which can be used to quantitate the amount of bound label.

The term "nucleic acid probe" refers to a nucleic acid molecule that binds to a specific sequence or sub-sequence of another nucleic acid molecule. A probe is preferably a nucleic acid molecule that binds through complementary base pairing to the full sequence or to a sub-sequence of a target nucleic acid. It will be understood that probes may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. Probes are preferably directly labelled as with isotopes, chromophores, lumiphores, chromogens, fluorescent proteins, or indirectly labelled such as with biotin to which a streptavidin complex may later bind. By assaying for the presence or absence of the probe, one can detect the presence or absence of the select sequence or sub-sequence.

A "labeled nucleic acid probe" is a nucleic acid probe that is bound, either covalently, through a linker, or through ionic, van der Waals or hydrogen bonds to a label such that the presence of the probe may be detected by detecting the presence of the label bound to the probe.

The terms "polypeptide" and "protein" refers to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The term "recombinant protein" refers to a protein that is produced by expression of a nucleotide sequence encoding the amino acid sequence of the protein from a recombinant DNA molecule.

The term "recombinant host cell" refers to a cell that comprises a recombinant nucleic acid molecule. Thus, for example, recombinant host cells can express genes that are not found within the native (non-recombinant) form of the cell.

The terms "isolated" "purified" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein or nucleic acid molecule which is the predominant protein or nucleic acid species present in a preparation is substantially purified. Generally, an isolated

protein or nucleic acid molecule will comprise more than 80% of all macromolecular species present in the preparation. Preferably, the protein is purified to represent greater than 90% of all macromolecular species present. More preferably the protein is purified to greater than 95%, and most preferably the protein is purified to essential homogeneity, wherein other macromolecular species are not detected by conventional techniques.

The term "naturally-occurring" as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "antibody" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, which specifically bind and recognize an analyte (antigen). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Antibodies exist, e.g., as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases. This includes, e.g., Fab' and $F(ab)'_2$ fragments. The term "antibody," as used herein, also includes antibody fragments either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA methodologies.

The term "immunoassay" refers to an assay that utilizes an antibody to specifically bind an analyte. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the analyte.

The term "identical" in the context of two nucleic acid or polypeptide sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. When percentage of sequence identity is used in reference to proteins or peptides it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acids residues are substituted for other amino acid residues with similar chemical properties (e.g. charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for

making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, *e.g.*, according to known algorithm. *See, e.g.*, Meyers and Miller, Computer Applic. Biol. Sci., 4: 11-17 (1988); Smith and Waterman (1981) *Adv. Appl. Math.* 2: 482; Needleman and Wunsch (1970) *J. Mol. Biol.* 48: 443; Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444; Higgins and Sharp (1988) *Gene*, 73: 237-244 and Higgins and Sharp (1989) *CABIOS* 5: 151-153; Corpet, *et al.* (1988) *Nucleic Acids Research* 16, 10881-90; Huang, *et al.* (1992) *Computer Applications in the Biosciences* 8, 155-65, and Pearson, *et al.* (1994) *Methods in Molecular Biology* 24, 307-31. Alignment is also often performed by inspection and manual alignment.

"Conservatively modified variations" of a particular nucleic acid sequence refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance, the codons CGU, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of "conservatively modified variations." Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques. Accordingly, each "silent variation" of a nucleic acid which encodes a polypeptide is implicit in each described sequence. Furthermore, one of skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are "conservatively modified variations" where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative amino acid substitutions

providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

The term "complementary" means that one nucleic acid molecule has the sequence of the binding partner of another nucleic acid molecule. Thus, the sequence 5'-ATGC-3' is complementary to the sequence 5'-GCAT-3'.

An amino acid sequence or a nucleotide sequence is "substantially identical" or "substantially similar" to a reference sequence if the amino acid sequence or nucleotide sequence has at least 80% sequence identity with the reference sequence over a given comparison window. Thus, substantially similar sequences include those having, for example, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity or at least 99% sequence identity. Two sequences that are identical to each other are, of course, also substantially identical.

A subject nucleotide sequence is "substantially complementary" to a reference nucleotide sequence if the complement of the subject nucleotide sequence is substantially identical to the reference nucleotide sequence.

The term "stringent conditions" refers to a temperature and ionic conditions used in nucleic acid hybridization. Stringent conditions are sequence dependent and are different under different environmental parameters. Generally, stringent conditions are selected to be about 5□C to 20□C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe.

The term "allelic variants" refers to polymorphic forms of a gene at a particular genetic locus, as well as cDNAs derived from mRNA transcripts of the genes and the polypeptides encoded by them.

The term "preferred mammalian codon" refers to the subset of codons from

among the set of codons encoding an amino acid that are most frequently used in proteins expressed in mammalian cells as chosen from the following list:

Amino Acid	Preferred codons for high level mammalian expression
Gly	GGC,GGG
Glu	GAG
Asp	GAC
Val	GUG,GUC
Ala	GCC,GCU
Ser	AGC,UCC
Lys	AAG
Asn	AAC
Met	AUG
Ile	AUC
Thr	ACC
Trp	UGG
Cys	UGC
Tyr	UAU,UAC
Leu	CUG
Phe	UUC
Arg	CGC,AGG,AGA
Gln	CAG
His	CAC
Pro	CCC

Fluorescent molecules are useful in fluorescence resonance energy transfer ("FRET"). FRET involves a donor molecule and an acceptor molecule. To optimize the efficiency and detectability of FRET between a donor and acceptor molecule, several factors need to be balanced. The emission spectrum of the donor should overlap as much as possible with the excitation spectrum of the acceptor to maximize the overlap integral. Also, the quantum yield of the donor moiety and the extinction coefficient of the acceptor should likewise be as high as possible to maximize R_o, the distance at which energy transfer efficiency is 50%. However, the excitation spectra of the donor and acceptor should overlap as little as possible so that a wavelength region can be found at which the donor can be excited efficiently without directly exciting the acceptor. Fluorescence arising from direct excitation of the acceptor is difficult to distinguish from fluorescence arising from FRET. Similarly, the emission spectra of the donor and acceptor should overlap as little as possible so that the two emissions can be clearly distinguished. High fluorescence quantum yield of

the acceptor moiety is desirable if the emission from the acceptor is to be measured either as the sole readout or as part of an emission ratio. One factor to be considered in choosing the donor and acceptor pair is the efficiency of fluorescence resonance energy transfer between them. Preferably, the efficiency of FRET between the donor and acceptor is at least 10%, more preferably at least 50% and even more preferably at least 80%.

The term "fluorescent property" refers to the molar extinction coefficient at an appropriate excitation wavelength, the fluorescence quantum efficiency, the shape of the excitation spectrum or emission spectrum, the excitation wavelength maximum and emission wavelength maximum, the ratio of excitation amplitudes at two different wavelengths, the ratio of emission amplitudes at two different wavelengths, the excited state lifetime, or the fluorescence anisotropy. A measurable difference in any one of these properties between wild-type *Aequorea* GFP and the mutant form is useful. A measurable difference can be determined by determining the amount of any quantitative fluorescent property, e.g., the amount of fluorescence at a particular wavelength, or the integral of fluorescence over the emission spectrum. Determining ratios of excitation amplitude or emission amplitude at two different wavelengths ("excitation amplitude ratioing" and "emission amplitude ratioing", respectively) are particularly advantageous because the ratioing process provides an internal reference and cancels out variations in the absolute brightness of the excitation source, the sensitivity of the detector, and light scattering or quenching by the sample.

II. LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS

A. Fluorescent Proteins

As used herein, the term "fluorescent protein" refers to any protein capable of fluorescence when excited with appropriate electromagnetic radiation. This includes fluorescent proteins whose amino acid sequences are either naturally occurring or engineered (i.e., analogs or mutants). Many cnidarians use green fluorescent proteins ("GFPs") as energy-transfer acceptors in bioluminescence. A "green fluorescent protein," as used herein, is a protein that fluoresces green light. Similarly, "blue fluorescent proteins" fluoresce blue light and "red fluorescent proteins" fluoresce red light. GFPs have been isolated from the Pacific Northwest jellyfish, *Aequorea victoria*, the sea pansy, *Renilla reniformis*, and *Phialidium gregarium*. W.W. Ward et al., *Photochem. Photobiol.*, 35:803-808 (1982); L.D. Levine et al., *Comp. Biochem. Physiol.*, 72B:77-85 (1982).

A variety of *Aequorea*-related fluorescent proteins having useful excitation and emission spectra have been engineered by modifying the amino acid sequence of a naturally occurring GFP from *Aequorea victoria*. (D.C. Prasher et al., *Gene*, 111:229-233 (1992); R. Heim et al., *Proc. Natl. Acad. Sci., USA*, 91:12501-04 (1994); U.S. patent application 08/337,915, filed November 10, 1994; International application PCT/US95/14692, filed 11/10/95.)

As used herein, a fluorescent protein is an "*Aequorea*-related fluorescent protein" if any contiguous sequence of 150 amino acids of the fluorescent protein has at least 85% sequence identity with an amino acid sequence, either contiguous or non-contiguous, from the 238 amino-acid wild-type *Aequorea* green fluorescent protein of Fig. 3 (SEQ ID NO:2). More preferably, a fluorescent protein is an *Aequorea*-related fluorescent protein if any contiguous sequence of 200 amino acids of the fluorescent protein has at least 95% sequence identity with an amino acid sequence, either contiguous or non-contiguous, from the wild type *Aequorea* green fluorescent protein of Fig. 3 (SEQ ID NO:2). Similarly, the fluorescent protein may be related to *Renilla* or *Phialidium* wild-type fluorescent proteins using the same standards.

Aequorea-related fluorescent proteins include, for example and without limitation, wild-type (native) *Aequorea victoria* GFP (D.C. Prasher et al., "Primary structure of the *Aequorea victoria* green fluorescent protein," *Gene*, (1992) 111:229-33), whose nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) are presented in Fig. 3; allelic variants of this sequence, e.g., Q80R, which has the glutamine

residue at position 80 substituted with arginine (M. Chalfie et al., *Science*, (1994) 263:802-805); those engineered *Aequorea*-related fluorescent proteins described herein, e.g., in Table A or Table F, variants that include one or more folding mutations and fragments of these proteins that are fluorescent, such as *Aequorea* green fluorescent protein from which the two amino-terminal amino acids have been removed. Several of these contain different aromatic amino acids within the central chromophore and fluoresce at a distinctly shorter wavelength than wild type species. For example, engineered proteins P4 and P4-3 contain (in addition to other mutations) the substitution Y66H, whereas W2 and W7 contain (in addition to other mutations) Y66W. Other mutations both close to the chromophore region of the protein and remote from it in primary sequence may affect the spectral properties of GFP and are listed in the first part of the table below.

TABLE A

Clone	Mutation(s)	Excitation max (nm)	Emission max (nm)	Extinct. Coeff. ($M^{-1}cm^{-1}$)	Quantum yield
Wild type	None	395 (475)	508	21,000 (7,150)	0.77
P4	Y66H	383	447	13,500	0.21
P4-3	Y66H Y145F	381	445	14,000	0.38
W7	Y66W N146I M153T V163A N212K	433 (453)	475 (501)	18,000 (17,100)	0.67
W2	Y66W I123V Y145H H148R M153T V163A N212K	432 (453)	480	10,000 (9,600)	0.72
S65T	S65T	489	511	39,200	0.68
P4-1	S65T M153A	504 (396)	514	14,500 (8,600)	0.53

	K238E		
S65A	S65A	471	504
S65C	S65C	479	507
S65L	S65L	484	510
Y66F	Y66F	360	442
Y66W	Y66W	458	480

Additional mutations in *Aequorea*-related fluorescent proteins, referred to as "folding mutations," improve the ability of fluorescent proteins to fold at higher temperatures, and to be more fluorescent when expressed in mammalian cells, but have little or no effect on the peak wavelengths of excitation and emission. It should be noted that these may be combined with mutations that influence the spectral properties of GFP to produce proteins with altered spectral and folding properties. Folding mutations include: F64L, V68L, S72A, and also T44A, F99S, Y145F, N146I, M153T or A, V163A, I167T, S175G, S205T and N212K.

As used herein, the term "loop domain" refers to an amino acid sequence of an *Aequorea*-related fluorescent protein that connects the amino acids involved in the secondary structure of the eleven strands of the □-barrel or the central □-helix (residues 56-72) (see Fig. 1A and 1B).

As used herein, the "fluorescent protein moiety" of a fluorescent protein is that portion of the amino acid sequence of a fluorescent protein which, when the amino acid sequence of the fluorescent protein substrate is optimally aligned with the amino acid sequence of a naturally occurring fluorescent protein, lies between the amino terminal and carboxy terminal amino acids, inclusive, of the amino acid sequence of the naturally occurring fluorescent protein.

It has been found that fluorescent proteins can be genetically fused to other target proteins and used as markers to identify the location and amount of the target protein produced. Accordingly, this invention provides fusion proteins comprising a fluorescent protein moiety and additional amino acid sequences. Such sequences can be, for example, up to about 15, up to about 50, up to about 150 or up to about 1000 amino acids long. The

fusion proteins possess the ability to fluoresce when excited by electromagnetic radiation. In one embodiment, the fusion protein comprises a polyhistidine tag to aid in purification of the protein.

B. Use Of The Crystal Structure Of Green Fluorescent Protein To Design Mutants Having Altered Fluorescent Characteristics

Using X-ray crystallography and computer processing, we have created a model of the crystal structure of *Aequorea* green fluorescent protein showing the relative location of the atoms in the molecule. This information is useful in identifying amino acids whose substitution alters fluorescent properties of the protein.

Fluorescent characteristics of *Aequorea*-related fluorescent proteins depend, in part, on the electronic environment of the chromophore. In general, amino acids that are within about 0.5 nm of the chromophore influence the electronic environment of the chromophore. Therefore, substitution of such amino acids can produce fluorescent proteins with altered fluorescent characteristics. In the excited state, electron density tends to shift from the phenolate towards the carbonyl end of the chromophore. Therefore, placement of increasing positive charge near the carbonyl end of the chromophore tends to decrease the energy of the excited state and cause a red-shift in the absorbance and emission wavelength maximum of the protein. Decreasing positive charge near the carbonyl end of the chromophore tends to have the opposte effect, causing a blue-shift in the protein's wavelengths.

Amino acids with charged (ionized D, E, K, and R), dipolar (H, N, Q, S, T, and uncharged D, E and K), and polarizable side groups (e.g., C, F, H, M, W and Y) are useful for altering the electronic environment of the chromophore, especially when they substitute an amino acid with an uncharged, nonpolar or non-polarizable side chain. In general, amino acids with polarizable side groups alter the electronic environment least, and; consequently, are expected to cause a comparatively smaller change in a fluorescent property. Amino acids with charged side groups alter the environment most, and, consequently, are expected to cause a comparatively larger change in a fluorescent property. However, amino acids with charged side groups are more likely to disrupt the structure of the protein and to prevent proper folding if buried next to the chromophore without any

additional solvation or salt bridging. Therefore charged amino acids are most likely to be tolerated and to give useful effects when they replace other charged or highly polar amino acids that are already solvated or involved in salt bridges. In certain cases, where substitution with a polarizable amino acid is chosen, the structure of the protein may make selection of a larger amino acid, e.g., W, less appropriate. Alternatively, positions occupied by amino acids with charged or polar side groups that are unfavorably oriented may be substituted with amino acids that have less charged or polar side groups. In another alternative, an amino acid whose side group has a dipole oriented in one direction in the protein can be substituted with an amino acid having a dipole oriented in a different direction.

More particularly, Table B lists several amino acids located within about 0.5 nm from the chromophore whose substitution can result in altered fluorescent characteristics. The table indicates, underlined, preferred amino acid substitutions at the indicated location to alter a fluorescent characteristic of the protein. In order to introduce such substitutions, the table also provides codons for primers used in site- directed mutagenesis involving amplification. These primers have been selected to encode economically the preferred amino acids, but they encode other amino acids as well, as indicated, or even a stop codon, denoted by Z. In introducing substitutions using such degenerate primers the most efficient strategy is to screen the collection to identify mutants with the desired properties and then sequence their DNA to find out which of the possible substitutions is responsible. Codons are shown in double-stranded form with sense strand above, antisense strand below. In nucleic acid sequences, R=(A or g); Y=(C or T); M=(A or C); K=(g or T); S=(g or C); W=(A or T); H=(A, T, or C); B=(g, T, or C); V=(g, A, or C); D=(g, A, or T); N=(A, C, g, or T).

TABLE B

Original position and presumed role		Change to	Codon
L42	Aliphatic residue near C=N of chromophore	CFHLQRWYZ	5'YDS 3' 3'RHS 5'
V61	Aliphatic residue near central -CH= of chromophore	FYHCLR	YDC RHg

T62	Almost directly above center of chromophore bridge	AVFS	KYC	MRg
		DEHKNQ	VAS	BTS
		FYHCLR	YDC	RHg
V68	Aliphatic residue near carbonyl and G67	FYHL	YWC	RWg
N121	Near C-N site of ring closure between T65 and G67	CFHLQRWYZ	YDS	RHS
Y145	Packs near tyrosine ring of chromophore	WCFL	TKS	AMS
		DEHNKQ	VAS	BTS
H148	H-bonds to phenolate oxygen	FYNI	WWC	WWg
		KQR	MRg	KYC
V150	Aliphatic residue near tyrosine ring of chromophore	FYHL	YWC	RWg
F165	Packs near tyrosine ring	CHQRWYZ	YRS	RYS
I167	Aliphatic residue near phenolate; I167T has effects	FYHL	YWC	RWg
T203	H-bonds to phenolic oxygen of chromophore	FHLQRWYZ	YDS	RHS
E222	Protonation regulates ionization of chromophore	HKNQ	MAS	KTS

Examples of amino acids with polar side groups that can be substituted with polarizable side groups include, for example, those in Table C.

TABLE C

Original position and presumed role			Change to	Codon
	Q69	Terminates chain of H-bonding waters	KREG	RRg YYC
	Q94	H-bonds to carbonyl terminus of chromophore	DEHKNQ	VAS BTS
	Q183	Bridges Arg96 and center of chromophore bridge	HY	YAC RTG
			EK	RAg YTC
	N185	Part of H-bond network near carbonyl of chromophore	DEHNKQ	VAS BTS

In another embodiment, an amino acid that is close to a second amino acid within about 0.5 nm of the chromophore can, upon substitution, alter the electronic properties of the second amino acid, in turn altering the electronic environment of the chromphore. Table D presents two such amino acids. The amino acids, L220 and V224, are close to E222 and oriented in the same direction in the □ pleated sheet.

TABLE D

Original position and presumed role			Change to	Codon
	L220	Packs next to Glu222; to make GFP pH sensitive	HKNPQT	MMS KKS
	V224	Packs next to Glu222; to make GFP pH sensitive	HKNPQT	MMS KKS
			CFHLQRWYZ	YDS RHS

One embodiment of the invention includes a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at Q69, wherein the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein. Preferably, the substitution at Q69 is selected from the group of K, R, E and G. The Q69 substitution can be combined with other mutations to improve the properties of the protein, such as a functional mutation at S65.

One embodiment of the invention includes a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at E222, but not including E222G, wherein the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein. Preferably, the substitution at E222 is selected from the group of N and Q. The E222 substitution can be combined with other mutations to improve the properties of the protein, such as a functional mutation at F64.

One embodiment of the invention includes a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at Y145, wherein the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

Preferably, the substitution at Y145 is selected from the group of W, C, F, L, E, H, K and Q. The Y145 substitution can be combined with other mutations to improve the properties of the protein, such as a Y66.

The invention also includes computer related embodiments, including computational methods of using the crystal coordinates for designing new fluorescent protein mutations and devices for storing the crystal data, including coordinates. For instance the invention includes a device comprising a storage device and, stored in the device, at least 10 atomic coordinates selected from the atomic coordinates listed in Figs. 5-1 to 5-28. More coordinates can be storage depending of the complexity of the calculations or the objective of using the coordinates (e.g. about 100, 1,000, or more coordinates). For example, larger numbers of coordinates will be desirable for more detailed representations of fluorescent protein structure. Typically, the storage device is a computer readable device that stores code that it receives as input the atomic coordinates. Although, other storage meand as known in the art are contemplated. The computer readable device can be a floppy disk or a hard drive.

C. Production Of Long Wavelength Engineered Fluorescent Proteins

Recombinant production of a fluorescent protein involves expressing a nucleic acid molecule having sequences that encode the protein.

In one embodiment, the nucleic acid encodes a fusion protein in which a single polypeptide includes the fluorescent protein moiety within a longer polypeptide. The longer polypeptide can include a second functional protein, such as FRET partner or a protein having a second function (e.g., an enzyme, antibody or other binding protein). Nucleic acids that encode fluorescent proteins are useful as starting materials.

The fluorescent proteins can be produced as fusion proteins by recombinant DNA technology. Recombinant production of fluorescent proteins involves expressing nucleic acids having sequences that encode the proteins. Nucleic acids encoding fluorescent proteins can be obtained by methods known in the art. Fluorescent proteins can be made by site-specific mutagenesis of other nucleic acids encoding fluorescent proteins, or by random mutagenesis caused by increasing the error rate of PCR of the original polynucleotide with 0.1 mM $MnCl_2$ and unbalanced nucleotide concentrations. See, *e.g.*, U.S. patent application 08/337,915, filed November 10, 1994 or International application PCT/US95/14692, filed 11/10/95. The nucleic acid encoding a green fluorescent protein can be isolated by polymerase chain reaction of cDNA from *A. victoria* using primers based on the DNA sequence of *A. victoria* green fluorescent protein, as presented in Fig. 3. PCR methods are described in, for example, U.S. Pat. No. 4,683,195; Mullis et al. (1987) *Cold Spring Harbor Symp. Quant. Biol.* 51:263; and Erlich, ed., *PCR Technology*, (Stockton Press, NY, 1989).

The construction of expression vectors and the expression of genes in transfected cells involves the use of molecular cloning techniques also well known in the art. Sambrook et al., *Molecular Cloning -- A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, (1989) and *Current Protocols in Molecular Biology*, F.M. Ausubel et al., eds., (Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc.). The expression vector can be adapted for function in prokaryotes or eukaryotes by inclusion of appropriate promoters, replication sequences, markers, etc.

Nucleic acids used to transfect cells with sequences coding for expression of the polypeptide of interest generally will be in the form of an expression vector including

expression control sequences operatively linked to a nucleotide sequence coding for expression of the polypeptide. As used, the term "nucleotide sequence coding for expression of" a polypeptide refers to a sequence that, upon transcription and translation of mRNA, produces the polypeptide. This can include sequences containing, *e.g.*, introns. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus, expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (*i.e.*, ATG) in front of a protein-encoding gene, splicing signals for introns, maintenance of the correct reading frame of that gene to permit proper translation of the mRNA, and stop codons.

Methods which are well known to those skilled in the art can be used to construct expression vectors containing the fluorescent protein coding sequence and appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. (See, for example, the techniques described in Maniatis, *et al., Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y., 1989).

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method by procedures well known in the art. Alternatively, $MgCl_2$ or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell or by electroporation.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransfected with DNA sequences encoding the fusion polypeptide of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (*Eukaryotic Viral*

Vectors, Cold Spring Harbor Laboratory, Gluzman ed., 1982). Preferably, a eukaryotic host is utilized as the host cell as described herein.

Techniques for the isolation and purification of either microbially or eukaryotically expressed polypeptides of the invention may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies or antigen.

In one embodiment recombinant fluorescent proteins can be produced by expression of nucleic acid encoding for the protein in *E. coli*. *Aequorea*-related fluorescent proteins are best expressed by cells cultured between about 15□ C and 30□ C but higher temperatures (e.g. 37□ C) are possible. After synthesis, these enzymes are stable at higher temperatures (e.g., 37□ C) and can be used in assays at those temperatures.

A variety of host-expression vector systems may be utilized to express fluorescent protein coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing a fluorescent protein coding sequence; yeast transformed with recombinant yeast expression vectors containing the fluorescent protein coding sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing a fluorescent protein coding sequence; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing a fluorescent protein coding sequence; or animal cell systems infected with recombinant virus expression vectors (*e.g.*, retroviruses, adenovirus, vaccinia virus) containing a fluorescent protein coding sequence, or transformed animal cell systems engineered for stable expression.

Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, *etc.* may be used in the expression vector (see, *e.g.*, Bitter, *et al.*, Methods in Enzymology 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage □, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. When cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the

retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the inserted fluorescent protein coding sequence.

In bacterial systems a number of expression vectors may be advantageously selected depending upon the use intended for the fluorescent protein expressed. For example, when large quantities of the fluorescent protein are to be produced, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Those which are engineered to contain a cleavage site to aid in recovering fluorescent protein are preferred.

In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review see, Current Protocols in Molecular Biology, Vol. 2, Ed. Ausubel, *et al.*, Greene Publish. Assoc. & Wiley Interscience, Ch. 13, 1988; Grant, *et al.*, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Eds. Wu & Grossman, 31987, Acad. Press, N.Y., Vol. 153, pp.516-544, 1987; Glover, DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3, 1986; and Bitter, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684, 1987; and The Molecular Biology of the Yeast Saccharomyces, Eds. Strathern *et al.*, Cold Spring Harbor Press, Vols. I and II, 1982. A constitutive yeast promoter such as ADH or LEU2 or an inducible promoter such as GAL may be used (Cloning in Yeast, Ch. 3, R. Rothstein In: DNA Cloning Vol.11, A Practical Approach, Ed. DM Glover, IRL Press, Wash., D.C., 1986). Alternatively, vectors may be used which promote integration of foreign DNA sequences into the yeast chromosome.

In cases where plant expression vectors are used, the expression of a fluorescent protein coding sequence may be driven by any of a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV (Brisson, *et al.*, *Nature* 310:511-514, 1984), or the coat protein promoter to TMV (Takamatsu, *et al.*, *EMBO J.* 6:307-311, 1987) may be used; alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi, *et al.*, 1984, *EMBO J.* 3:1671-1680; Broglie, *et al.*, *Science* 224:838-843, 1984); or heat shock promoters, *e.g.*, soybean hsp17.5-E or hsp17.3-B (Gurley, *et al.*, *Mol. Cell. Biol.* 6:559-565, 1986) may be used. These constructs can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation,

microinjection, electroporation, *etc.* For reviews of such techniques see, for example, Weissbach & Weissbach, *Methods for Plant Molecular Biology*, Academic Press, NY, Section VIII, pp. 421-463, 1988; and Grierson & Corey, *Plant Molecular Biology*, 2d Ed., Blackie, London, Ch. 7-9, 1988.

An alternative expression system which could be used to express fluorescent protein is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The fluorescent protein coding sequence may be cloned into non-essential regions (for example, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the fluorescent protein coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed, see Smith, *et al., J. Viol.* 46:584, 1983; Smith, U.S. Patent No. 4,215,051.

Eukaryotic systems, and preferably mammalian expression systems, allow for proper post-translational modifications of expressed mammalian proteins to occur. Eukaryotic cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, phosphorylation, and, advantageously secretion of the gene product should be used as host cells for the expression of fluorescent protein. Such host cell lines may include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, Jurkat, HEK-293, and WI38.

Mammalian cell systems which utilize recombinant viruses or viral elements to direct expression may be engineered. For example, when using adenovirus expression vectors, the fluorescent protein coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the fluorescent protein in infected hosts (*e.g.*, see Logan & Shenk, *Proc. Natl. Acad. Sci. USA*, 81: 3655-3659, 1984). Alternatively, the vaccinia virus 7.5K promoter may be used. (*e.g.*, see, Mackett, *et al.*, *Proc. Natl. Acad. Sci. USA* ,79: 7415-7419, 1982; Mackett, *et al., J.*

Virol. 49: 857-864, 1984; Panicali, *et al.*, *Proc. Natl. Acad. Sci. USA* 79: 4927-4931, 1982). Of particular interest are vectors based on bovine papilloma virus which have the ability to replicate as extrachromosomal elements (Sarver, *et al.*, *Mol. Cell. Biol.* 1: 486, 1981). Shortly after entry of this DNA into mouse cells, the plasmid replicates to about 100 to 200 copies per cell. Transcription of the inserted cDNA does not require integration of the plasmid into the host's chromosome, thereby yielding a high level of expression. These vectors can be used for stable expression by including a selectable marker in the plasmid, such as the *neo* gene. Alternatively, the retroviral genome can be modified for use as a vector capable of introducing and directing the expression of the fluorescent protein gene in host cells (Cone & Mulligan, *Proc. Natl. Acad. Sci. USA*, 81:6349-6353, 1984). High level expression may also be achieved using inducible promoters, including, but not limited to, the metallothionine IIA promoter and heat shock promoters.

The invention can also include a localization sequence, such as a nuclear localization sequence, an endoplasmic reticulum localization sequence, a peroxisome localization sequence, a mitochondrial localization sequence, or a localized protein. Localization sequences can be targeting sequences which are described, for example, in "Protein Targeting", chapter 35 of Stryer, L., *Biochemistry* (4th ed.). W.H. Freeman, 1995. The localization sequence can also be a localized protein. Some important localization sequences include those targeting the nucleus (KKKRK), mitochondrion (amino terminal MLRTSSLFTRRVQPSLFRNILRLQST-), endoplasmic reticulum (KDEL at C-terminus, assuming a signal sequence present at N-terminus), peroxisome (SKF at C-terminus), prenylation or insertion into plasma membrane (CaaX, CC, CXC, or CCXX at C-terminus), cytoplasmic side of plasma membrane (fusion to SNAP-25), or the Golgi apparatus (fusion to furin).

For long-term, high-yield production of recombinant proteins, stable expression is preferred. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with the fluorescent protein cDNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. For example, following the introduction of foreign DNA,

engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, *et al.*, *Cell*, 11: 223, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA*, 48:2026, 1962), and adenine phosphoribosyltransferase (Lowy, *et al.*, *Cell*, 22: 817, 1980) genes can be employed in tk$^-$, hgprt$^-$ or aprt$^-$ cells respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, *et al.*, *Proc. Natl. Acad. Sci. USA*, 77: 3567, 1980; O'Hare, *et al.*, *Proc. Natl. Acad. Sci. USA*, 8: 1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA*, 78: 2072, 1981; neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, *J. Mol. Biol.*, 150:1, 1981); and hygro, which confers resistance to hygromycin (Santerre, *et al.*, *Gene*, 30: 147, 1984) genes. Recently, additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. USA*, 85:8047, 1988); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue L., In: *Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory, ed., 1987).

DNA sequences encoding the fluorescence protein polypeptide of the invention can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, in other words when the foreign DNA is continuously maintained in the host, are known in the art.

The expression vector can be transfected into a host cell for expression of the recombinant nucleic acid. Host cells can be selected for high levels of expression in order to purify the fluorescent protein fusion protein. *E. coli* is useful for this purpose. Alternatively, the host cell can be a prokaryotic or eukaryotic cell selected to study the activity of an enzyme produced by the cell. In this case, the linker peptide is selected to

include an amino acid sequence recognized by the protease. The cell can be, *e.g.*, a cultured cell or a cell *in vivo*.

A primary advantage of fluorescent protein fusion proteins is that they are prepared by normal protein biosynthesis, thus completely avoiding organic synthesis and the requirement for customized unnatural amino acid analogs. The constructs can be expressed in *E. coli* in large scale for *in vitro* assays. Purification from bacteria is simplified when the sequences include polyhistidine tags for one-step purification by nickel-chelate chromatography. Alternatively, the substrates can be expressed directly in a desired host cell for assays *in situ*.

In another embodiment, the invention provides a transgenic non-human animal that expresses a nucleic acid sequence which encodes the fluorescent protein.

The "non-human animals" of the invention comprise any non-human animal having nucleic acid sequence which encodes a fluorescent protein. Such non-human animals include vertebrates such as rodents, non-human primates, sheep, dog, cow, pig, amphibians, and reptiles. Preferred non-human animals are selected from the rodent family including rat and mouse, most preferably mouse. The "transgenic non-human animals" of the invention are produced by introducing "transgenes" into the germline of the non-human animal. Embryonal target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonal target cell. The zygote is the best target for micro-injection. In the mouse, the male pronucleus reaches the size of approximately 20 micrometers in diameter which allows reproducible injection of 1-2 pl of DNA solution. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host gene before the first cleavage (Brinster *et al., Proc. Natl. Acad. Sci. USA* 82:4438-4442, 1985). As a consequence, all cells of the transgenic non-human animal will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene. Microinjection of zygotes is the preferred method for incorporating transgenes in practicing the invention.

The term "transgenic" is used to describe an animal which includes exogenous genetic material within all of its cells. A "transgenic" animal can be produced by cross-breeding two chimeric animals which include exogenous genetic material within cells used

in reproduction. Twenty-five percent of the resulting offspring will be transgenic *i.e.*, animals which include the exogenous genetic material within all of their cells in both alleles. 50% of the resulting animals will include the exogenous genetic material within one allele and 25% will include no exogenous genetic material.

Retroviral infection can also be used to introduce transgene into a non-human animal. The developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, the blastomeres can be targets for retro viral infection (Jaenich, R., *Proc. Natl. Acad. Sci USA* 73:1260-1264, 1976). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan, *et al.* (1986) in Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The viral vector system used to introduce the transgene is typically a replication-defective retro virus carrying the transgene (Jahner, *et al.*, *Proc. Natl. Acad. Sci. USA* 82:6927-6931, 1985; Van der Putten, *et al.*, *Proc. Natl. Acad. Sci USA* 82:6148-6152, 1985). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten, *supra*; Stewart, *et al.*, *EMBO J.* 6:383-388, 1987). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (D. Jahner *et al.*, *Nature* 298:623-628, 1982). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic nonhuman animal. Further, the founder may contain various retro viral insertions of the transgene at different positions in the genome which generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line, albeit with low efficiency, by intrauterine retro viral infection of the midgestation embryo (D. Jahner *et al.*, *supra*).

A third type of target cell for transgene introduction is the embryonal stem cell (ES). ES cells are obtained from pre-implantation embryos cultured *in vitro* and fused with embryos (M. J. Evans *et al. Nature* 292:154-156, 1981; M.O. Bradley *et al.*, *Nature* 309: 255-258, 1984; Gossler, *et al.*, *Proc. Natl. Acad. Sci USA* 83: 9065-9069, 1986; and Robertson *et al.*, *Nature* 322:445-448, 1986). Transgenes can be efficiently introduced into the ES cells by DNA transfection or by retro virus-mediated transduction. Such transformed ES cells can thereafter be combined with blastocysts from a nonhuman animal. The ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal. (For review see Jaenisch, R., *Science* 240: 1468-1474, 1988).

"Transformed" means a cell into which (or into an ancestor of which) has been introduced, by means of recombinant nucleic acid techniques, a heterologous nucleic acid molecule. "Heterologous" refers to a nucleic acid sequence that either originates from another species or is modified from either its original form or the form primarily expressed in the cell.

"Transgene" means any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism (*i.e.*, either stably integrated or as a stable extrachromosomal element) which develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (*i.e.*, foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism. Included within this definition is a transgene created by the providing of an RNA sequence which is transcribed into DNA and then incorporated into the genome. The transgenes of the invention include DNA sequences which encode which encodes the fluorescent protein which may be expressed in a transgenic non-human animal. The term "transgenic" as used herein additionally includes any organism whose genome has been altered by *in vitro* manipulation of the early embryo or fertilized egg or by any transgenic technology to induce a specific gene knockout. The term "gene knockout" as used herein, refers to the targeted disruption of a gene *in vivo* with complete loss of function that has been achieved by any transgenic technology familiar to those in the art. In one embodiment, transgenic animals having gene knockouts are those in which the target gene has been rendered nonfunctional by an insertion targeted to the gene to be rendered non-functional by homologous recombination. As used herein, the term "transgenic" includes any transgenic technology familiar to those in the art which can produce an organism carrying an introduced transgene or one in which an endogenous gene has been rendered non-functional or "knocked out."

III. USES OF ENGINEERED FLUORESCENT PROTEINS

The proteins of this invention are useful in any methods that employ fluorescent proteins.

The engineered fluorescent proteins of this invention are useful as fluorescent markers in the many ways fluorescent markers already are used. This includes, for example, coupling engineered fluorescent proteins to antibodies, nucleic acids or other receptors for use in detection assays, such as immunoassays or hybridization assays.

The engineered fluorescent proteins of this invention are useful to track the movement of proteins in cells. In this embodiment, a nucleic acid molecule encoding the fluorescent protein is fused to a nucleic acid molecule encoding the protein of interest in an expression vector. Upon expression inside the cell, the protein of interest can be localized based on fluorescence. In another version, two proteins of interest are fused with two engineered fluorescent proteins having different fluorescent characteristics.

The engineered fluorescent proteins of this invention are useful in systems to detect induction of transcription. In certain embodiments, a nucleotide sequence encoding the engineered fluorescent protein is fused to expression control sequences of interest and the expression vector is transfected into a cell. Induction of the promoter can be measured by detecting the expression and/or quantity of fluorescence. Such constructs can be used used to follow signaling pathways from receptor to promoter.

The engineered fluorescent proteins of this invention are useful in applications involving FRET. Such applications can detect events as a function of the movement of fluorescent donors and acceptor towards or away from each other. One or both of the donor/acceptor pair can be a fluorescent protein. A preferred donor and receptor pair for FRET based assays is a donor with a T203I mutation and an acceptor with the mutation T203X, wherein X is an aromatic amino acid-39, especially T203Y, T203W, or T203H. In a particularly useful pair the donor contains the following mutations: S72A, K79R, Y145F, M153A and T203I (with a excitation peak of 395 nm and an emission peak of 511 nm) and the acceptor contains the following mutations S65G, S72A, K79R, and T203Y. This particular pair provides a wide separation between the excitation and emission peaks of the donor and provides good overlap between the donor emission spectrum and the acceptor excitation spectrum. Other red-shifted mutants, such as those described herein, can also be used as the acceptor in such a pair.

In one aspect, FRET is used to detect the cleavage of a substrate having the donor and acceptor coupled to the substrate on opposite sides of the cleavage site. Upon cleavage of the substrate, the donor/acceptor pair physically separate, eliminating FRET. Assays involve contacting the substrate with a sample, and determining a qualitative or quantitative change in FRET. In one embodiment, the engineered fluorescent protein is used in a substrate for □-lactamase. Examples of such substrates are described in United States patent applications 08/407,544, filed March 20, 1995 and International Application

PCT/US96/04059, filed March 20, 1996. In another embodiment, an engineered fluorescent protein donor/acceptor pair are part of a fusion protein coupled by a peptide having a proteolytic cleavage site. Such tandem fluorescent proteins are described in United States patent application 08/594,575, filed January 31, 1996.

In another aspect, FRET is used to detect changes in potential across a membrane. A donor and acceptor are placed on opposite sides of a membrane such that one translates across the membrane in response to a voltage change. This creates a measurable FRET. Such a method is described in United States patent application 08/481,977, filed June 7, 1995 and International Application PCT/US96/09652, filed June 6, 1996.

The engineered protein of this invention are useful in the creation of fluorescent substrates for protein kinases. Such substrates incorporate an amino acid sequence recognizable by protein kinases. Upon phosphorylation, the engineered fluorescent protein undergoes a change in a fluorescent property. Such substrates are useful in detecting and measuring protein kinase activity in a sample of a cell, upon transfection and expression of the substrate. Preferably, the kinase recognition site is placed within about 20 amino acids of a terminus of the engineered fluorescent protein. The kinase recognition site also can be placed in a loop domain of the protein. (See, e.g. Figure 1B.) Methods for making fluorescent substrates for protein kinases are described in United States patent application 08/680,877, filed July 16, 1996.

A protease recognition site also can be introduced into a loop domain. Upon cleavage, fluorescent property changes in a measurable fashion.

The invention also includes a method of identifying a test chemical. Typically, the method includes contacting a test chemical a sample containing a biological entity labeled with a functional, engineered fluorescent protein or a polynucleotide encoding said functional, engineered fluorescent protein. By monitoring fluorescence (i.e. a fluorescent property) from the sample containing the functional engineered fluorescent protein it can be determined whether a test chemical is active. Controls can be included to insure the specificity of the signal. Such controls include measurements of a fluorescent property in the absence of the test chemical, in the presence of a chemical with an expected activity (e.g., a known modulator) or engineered controls (e.g., absence of engineered fluorescent protein, absence of engineered fluorescent protein polynucleotide or the absence of operably linkage of the engineered fluorescent protein).

The fluorescence in the presence of a test chemical can be greater or less than in the absence of said test chemical. For instance if the engineered fluorescent protein is used a reporter of gene expression, the test chemical may up or down regulate gene expression. For such types of screening, the polynucleotide encoding the functional, engineered fluorescent protein is operatively linked to a genomic polynucleotide or a re. Alternatively, the functional, engineered fluorescent protein is fused to second functional protein. This embodiment can be used to track localization of the second protein or to track protein-protein interactions using energy transfer.

IV. PROCEDURES

Fluorescence in a sample is measured using a fluorimeter. In general, excitation radiation from an excitation source having a first wavelength, passes through excitation optics. The excitation optics cause the excitation radiation to excite the sample. In response, fluorescent proteins in the sample emit radiation which has a wavelength that is different from the excitation wavelength. Collection optics then collect the emission from the sample. The device can include a temperature controller to maintain the sample at a specific temperature while it is being scanned. According to one embodiment, a multi-axis translation stage moves a microtiter plate holding a plurality of samples in order to position different wells to be exposed. The multi-axis translation stage, temperature controller, auto-focusing feature, and electronics associated with imaging and data collection can be managed by an appropriately programmed digital computer. The computer also can

transform the data collected during the assay into another format for presentation. This process can be miniaturized and automated to enable screening many thousands of compounds.

Methods of performing assays on fluorescent materials are well known in the art and are described in, e.g., Lakowicz, J.R., *Principles of Fluorescence Spectroscopy*, New York:Plenum Press (1983); Herman, B., Resonance energy transfer microscopy, in: *Fluorescence Microscopy of Living Cells in Culture, Part B, Methods in Cell Biology*, vol. 30, ed. Taylor, D.L. & Wang, Y.-L., San Diego: Academic Press (1989), pp. 219-243; Turro, N.J., *Modern Molecular Photochemistry*, Menlo Park: Benjamin/Cummings Publishing Col, Inc. (1978), pp. 296-361.

The following examples are provided by way of illustration, not by way of limitation.

EXAMPLES

As a step in understanding the properties of GFP, and to aid in the tailoring of GFPs with altered characteristics, we have determined the three dimensional structure at 1.9 Å resolution of the S65T mutant (R. Heim et al. *Nature* 373:664-665 (1995)) of *A. victoria* GFP. This mutant also contains the ubiquitous Q80R substitution, which accidentally occurred in the early distribution of the GFP cDNA and is not known to have any effect on the protein properties (M. Chalfie et al. *Science* 263:802-805 (1994)).

Histidine-tagged S65T GFP (R. Heim et al. *Nature* 373:664-665 (1995)) was overexpressed in JM109/pRSET$_B$ in 4 l YT broth plus ampicillin at 37□, 450 rpm and 5 l/min air flow. The temperature was reduced to 25□ at A_{595} = 0.3, followed by induction with 1mM isopropylthiogalactoside for 5h. Cell paste was stored at -80□ overnight, then was resuspended in 50 mM HEPES pH 7.9, 0.3 M NaCl, 5 mM 2-mercaptoethanol, 0.1 mM phenylmethyl-sulfonylfluoride (PMSF), passed once through a French press at 10,000 psi, then centrifuged at 20 K rpm for 45 min. The supernatant was applied to a Ni-NTA-agarose column (Qiagen), followed by a wash with 20 mM imidazole, then eluted with 100 mM imidazole. Green fractions were pooled and subjected to chymotryptic (Sigma) proteolysis (1:50 w/w) for 22 h at RT. After addition of 0.5 mM PMSF, the digest was reapplied to the

Ni column. N-terminal sequencing verified the presence of the correct N-terminal methionine. After dialysis against 20 mM HEPES, pH 7.5 and concentration to $A_{490} = 20$, rod-shaped crystals were obtained at RT in hanging drops containing 5 □l protein and 5 □l well solution, 22-26% PEG 4000 (Serva), 50 mM HEPES pH 8.0-8.5, 50 mM $MgCl_2$ and 10 mM 2-mercapto-ethanol within 5 days. Crystals were 0.05 mm across and up to 1.0 mm long. The space group is $P2_12_12_1$ with a = 51.8, b = 62.8, c = 70.7 Å, Z=4. Two crystal forms of wild-type GFP, unrelated to the present form, have been described by M. A. Perrozo, K. B. Ward, R. B. Thompson, & W. W. Ward. *J. Biol. Chem.* 203, 7713-7716 (1988).

The structure of GFP was determined by multiple isomorphous replacement and anomalous scattering (Table E), solvent flattening, phase combination and crystallographic refinement. The most remarkable feature of the fold of GFP is an eleven stranded ß-barrel wrapped around a single central helix (Fig. 1A and 1B), where each strand consists of approximately 9-13 residues. The barrel forms a nearly perfect cylinder 42 Å long and 24 Å in diameter. The N-terminal half of the polypeptide comprises three anti-parallel strands, the central helix, and then 3 more anti-parallel strands, the latter of which (residues 118-123) is parallel to the N-terminal strand (residues 11-23). The polypeptide backbone then crosses the "bottom" of the molecule to form the second half of the barrel in a five-strand Greek Key motif. The top end of the cylinder is capped by three short, distorted helical segments, while one short, very distorted helical segment caps the bottom of the cylinder. The main-chain hydrogen bonding lacing the surface of the cylinder very likely accounts for the unusual stability of the protein towards denaturation and proteolysis. There are no large segments of the polypeptide that could be excised while preserving the intactness of the shell around the chromophore. Thus it would seem difficult to re-engineer GFP to reduce its molecular weight (J. Dopf & T.M. Horiagon *Gene* 173:39-43 (1996)) by a large percentage.

The *p*-hydroxybenzylideneimidazolidinone chromophore (C. W. Cody et al. *Biochemistry* 32:1212-1218 (1993)) is completely protected from bulk solvent and centrally located in the molecule. The total and presumably rigid encapsulation is probably responsible for the small Stokes' shift (i.e. wavelength difference between excitation and emission maxima), high quantum yield of fluorescence, inability of O_2 to quench the excited state (B.D. Nageswara Rao et al. *Biophys. J.* 32:630-632 (1980)), and resistance of the

chromophore to titration of the external pH (W. W. Ward. *Bioluminescence and Chemiluminescence* (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman. *Biochemistry* 21:4535-4540 (1982); W. W. Ward et al. *Photochem. Photobiol.* 35:803-808 (1982)). It also allows one to rationalize why fluorophore formation should be a spontaneous intramolecular process (R. Heim et al. *Proc. Natl. Acad. Sci.* USA 91:12501-12504 (1994)), as it is difficult to imagine how an enzyme could gain access to the substrate. The plane of the chromophore is roughly perpendicular (60□) to the symmetry axis of the surrounding barrel. One side of the chromophore faces a surprisingly large cavity, that occupies a volume of approximately 135 $Å^3$ (B. Lee & F. M. Richards. *J. Mol. Biol.* 55:379-400 (1971)). The atomic radii were those of Lee & Richards, calculated using the program MS with a probe radius of 1.4 Å. (M. L. Connolly, *Science* 221:709-713 (1983)). The cavity does not open out to bulk solvent. Four water molecules are located in the cavity, forming a chain of hydrogen bonds linking the buried side chains of Glu^{222} and Gln^{69}. Unless occupied, such a large cavity would be expected to de-stabilize the protein by several kcal/mol (S. J. Hubbard et al., *Protein Engineering* 7:613-626 (1994); A. E. Eriksson et al. *Science* 255:178-183 (1992)). Part of the volume of the cavity might be the consequence of the compaction resulting from cyclization and dehydration reactions. The cavity might also temporarily accommodate the oxidant, most likely O_2 (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995); R. Heim et al. *Proc. Natl. Acad. Sci.* USA 91:12501-12504 (1994); S. Inouye & F.I. Tsuji. *FEBS Lett.* 351:211-214 (1994)), that dehydrogenates the □-□ bond of Tyr^{66}. The chromophore, cavity, and side chains that contact the chromophore are shown in Figure 2A and a portion of the final electron density map in this vicinity in 2B.

The opposite side of the chromophore is packed against several aromatic and polar side chains. Of particular interest is the intricate network of polar interactions with the chromophore (Fig. 2C). His^{148}, Thr^{203} and Ser^{205} form hydrogen bonds with the phenolic hydroxyl; Arg^{96} and Gln^{94} interact with the carbonyl of the imidazolidinone ring and Glu^{222} forms a hydrogen bond with the side chain of Thr^{65}. Additional polar interactions, such as hydrogen bonds to Arg^{96} from the carbonyl of Thr^{62}, and the side-chain carbonyl of Gln^{183}, presumably stabilize the buried Arg^{96} in its protonated form. In turn, this buried charge suggests that a partial negative charge resides on the carbonyl oxygen of the imidazolidinone ring of the deprotonated fluorophore, as has previously been suggested (W.

W. Ward. *Bioluminescence and Chemiluminescence* (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman. *Biochemistry* 21:4535-4540 (1982); W. W. Ward et al. *Photochem. Photobiol.* 35:803-808 (1982)). Arg^{96} is likely to be essential for the formation of the fluorophore, and may help catalyze the initial ring closure. Finally, Tyr^{145} shows a typical stabilizing edge-face interaction with the benzyl ring. Trp^{57}, the only tryptophan in GFP, is located 13 Å to 15 Å from the chromophore and the long axes of the two ring systems are nearly parallel. This indicates that efficient energy transfer to the latter should occur, and explains why no separate tryptophan emission is observable (D.C. Prasher et al. *Gene* 111:229-233 (1992). The two cysteines in GFP, Cys^{48} and Cys^{70}, are 24 Å apart, too distant to form a disulfide bridge. Cys^{70} is buried, but Cys^{48} should be relatively accessible to sulfhydryl-specific reagents. Such a reagent, 5,5'-dithiobis(2-nitrobenzoic acid), is reported to label GFP and quench its fluorescence (S. Inouye & F.I. Tsuji *FEBS Lett.* 351:211-214 (1994)). This effect was attributed to the necessity for a free sulfhydryl, but could also reflect specific quenching by the 5-thio-2-nitrobenzoate moiety that would be attached to Cys^{48}.

Although the electron density map is for the most part consistent with the proposed structure of the chromophore (D.C. Prasher et al. *Gene* 111:229-233 (1992); C. W. Cody et al. *Biochemistry* 32:1212-1218 (1993)) in the *cis* [Z-] configuration, with no evidence for any substantial fraction of the opposite isomer around the chromophore double bond, difference features are found at >4 □ in the final (F_o-F_c) electron density map that can be interpreted to represent either the intact, uncyclized polypeptide or a carbinolamine (inset to Fig. 2C). This suggests that a significant fraction, perhaps as much as 30% of the molecules in the crystal, have failed to undergo the final dehydration reaction.
Confirmation of incomplete dehydration comes from electrospray mass spectrometry, which consistently shows that the average masses of both wild-type and S65T GFP (31,086±4 and 31,099.5±4 Da, respectively) are 6-7 Da higher than predicted (31,079 and 31,093 Da, respectively) for the fully matured proteins. Such a discrepancy could be explained by a 30-35% mole fraction of apoprotein or carbinolamine with 18 or 20 Da higher molecular weight The natural abundance of ^{13}C and ^{2}H and the finite resolution of the Hewlett-Packard 5989B electrospray mass spectrometer used to make these measurements do not permit the individual peaks to be resolved, but instead yields an average mass peak with a full width at half maximum of approximately 15 Da. The molecular weights shown include the His-tag,

which has the sequence MRGSHHHHHH GMASMTGGQQM GRDLYDDDDK DPPAEF (SEQ ID NO:5). Mutants of GFP that increase the efficiency of fluorophore maturation might yield somewhat brighter preparations. In a model for the apoprotein, the Thr^{65}-Tyr^{66} peptide bond is approximately in the □-helical conformation, while the peptide of Tyr^{66}-Gly^{67} appears to be tipped almost perpendicular to the helix axis by its interaction with Arg^{96}. This further supports the speculation that Arg^{96} is important in generating the conformation required for cyclization, and possibly also for promoting the attack of Gly^{67} on the carbonyl carbon of Thr^{65} (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995)).

The results of previous random mutagenesis have implicated several amino acid side chains to have substantial effects on the spectra and the atomic model confirms that these residues are close to the chromophore. The mutations T203I and E222G have profound but opposite consequences on the absorption spectrum (T. Ehrig et al. *FEBS Letters* 367:163-166 (1995)). T203I (with wild-type Ser^{65}) lacks the 475 nm absorbance peak usually attributed to the anionic chromophore and shows only the 395 nm peak thought to reflect the neutral chromophore (R. Heim et al. *Proc. Natl. Acad. Sci.* USA 91:12501-12504 (1994); T. Ehrig et al. *FEBS Letters* 367:163-166 (1995)). Indeed, Thr^{203} is hydrogen-bonded to the phenolic oxygen of the chromophore, so replacement by Ile should hinder ionization of the phenolic oxygen. Mutation of Glu^{222} to Gly (T. Ehrig et al. *FEBS Letters* 367:163-166 (1995)) has much the same spectroscopic effect as replacing Ser^{65} by Gly, Ala, Cys, Val, or Thr, namely to suppress the 395 nm peak in favor of a peak at 470-490 nm (R. Heim et al. *Nature* 373:664-665 (1995); S. Delagrave et al. *Bio/Technology* 13:151-154 (1995)). Indeed Glu^{222} and the remnant of Thr^{65} are hydrogen-bonded to each other in the present structure, probably with the uncharged carboxyl of Glu^{222} acting as donor to the side chain oxygen of Thr^{65}. Mutations E222G, S65G, S65A, and S65V would all suppress such H-bonding. To explain why only wild-type protein has both excitation peaks, Ser^{65}, unlike Thr^{65}, may adopt a conformation in which its hydroxyl donates a hydrogen bond to and stabilizes Glu^{222} as an anion, whose charge then inhibits ionization of the chromophore. The structure also explains why some mutations seem neutral. For example, Gln^{80} is a surface residue far removed from the chromophore, which explains why its accidental and ubiquitous mutation to Arg seems to have no obvious intramolecular spectroscopic effect (M. Chalfie et al. *Science* 263:802-805 (1994)).

The development of GFP mutants with red-shifted excitation and emission

maxima is an interesting challenge in protein engineering (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995); R. Heim et al. *Nature* 373:664-665 (1995); S. Delagrave et al. *Bio/Technology* 13:151-154 (1995)). Such mutants would also be valuable for avoidance of cellular autofluorescence at short wavelengths, for simultaneous multicolor reporting of the activity of two or more cellular processes, and for exploitation of fluorescence resonance energy transfer as a signal of protein-protein interaction (R. Heim & R.Y. Tsien. *Current Biol.* 6:178-182 (1996)). Extensive attempts using random mutagenesis have shifted the emission maximum by at most 6 nm to longer wavelengths, to 514 nm (R. Heim & R.Y. Tsien. *Current Biol.* 6:178-182 (1996)); previously described "red-shifted" mutants merely suppressed the 395 nm excitation peak in favor of the 475 nm peak without any significant reddening of the 505 nm emission (S. Delagrave et al. *Bio/Technology* 13:151-154 (1995)). Because Thr^{203} is revealed to be adjacent to the phenolic end of the chromophore, we mutated it to polar aromatic residues such as His, Tyr, and Trp in the hope that the additional polarizability of their □ systems would lower the energy of the excited state of the adjacent chromophore. All three substitutions did indeed shift the emission peak to greater than 520 nm (Table F). A particularly attractive mutation was T203Y/S65G/V68L/S72A, with excitation and emission peaks at 513 and 527 nm respectively. These wavelengths are sufficiently different from previous GFP mutants to be readily distinguishable by appropriate filter sets on a fluorescence microscope. The extinction coefficient, 36,500 $M^{-1}cm^{-1}$, and quantum yield, 0.63, are almost as high as those of S65T (R. Heim et al. *Nature* 373:664-665 (1995)).

Comparison of *Aequorea* GFP with other protein pigments is instructive. Unfortunately, its closest characterized homolog, the GFP from the sea pansy *Renilla reniformis* (O. Shimomura and F.H. Johnson *J. Cell. Comp. Physiol.* 59:223 (1962); J. G. Morin and J. W. Hastings, *J. Cell. Physiol.* 77:313 (1971); H. Morise et al. *Biochemistry* 13:2656 (1974); W. W. Ward *Photochem. Photobiol. Reviews* (Smith, K. C. ed.) 4:1 (1979); W. W. Ward. *Bioluminescence and Chemiluminescence* (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman *Biochemistry* 21:4535-4540 (1982); W. W. Ward et al. *Photochem. Photobiol.* 35:803-808 (1982)), has not been sequenced or cloned, though its chromophore is derived from the same FSYG sequence as in wild-type *Aequorea* GFP (R. M. San Pietro et al. *Photochem. Photobiol.* 57:63S (1993)). The closest analog for which a three dimensional structure is

available is the photoactive yellow protein (PYP, G. E. O. Borgstahl et al. *Biochemistry* 34:6278-6287 (1995)), a 14-kDa photoreceptor from halophilic bacteria. PYP in its native dark state absorbs maximally at 446 nm and transduces light with a quantum yield of 0.64, rather closely matching wild-type GFP's long wavelength absorbance maximum near 475 nm and fluorescence quantum yield of 0.72-0.85. The fundamental chromophore in both proteins is an anionic *p*-hydroxycinnamyl group, which is covalently attached to the protein via a thioester linkage in PYP and a heterocyclic iminolactam in GFP. Both proteins stabilize the negative charge on the chromophore with the help of buried cationic arginine and neutral glutamic acid groups, Arg^{52} and Glu^{46} in PYP and Arg^{96} and Glu^{222} in GFP, though in PYP the residues are close to the oxyphenyl ring whereas in GFP they are nearer the carbonyl end of the chromophore. However, PYP has an overall □/□ fold with appropriate flexibility and signal transduction domains to enable it to mediate the cellular phototactic response, whereas GFP is a much more regular and rigid □-barrel to minimize parasitic dissipation of the excited state energy as thermal or conformational motions. GFP is an elegant example of how a visually appealing and extremely useful function, efficient fluorescence, can be spontaneously generated from a cohesive and economical protein structure.

A. Summary Of GFP Structure Determination

Data were collected at room temperature in house using either Molecular Structure Corp. R-axis II or San Diego Multiwire Systems (SDMS) detectors (Cu K□) and later at beamline X4A at the Brookhaven National Laboratory at the selenium absorption edge (□ = 0.979 Å) using image plates. Data were evaluated using the HKL package (Z. Otwinowski, in *Proceedings of the CCP4 Study Weekend: Data Collection and Processing*, L. Sawyer, N. Issacs, S. Bailey, Eds. (Science and Engineering Research Council (SERC), Daresbury Laboratory, Warrington, UK, (1991)), pp 56-62; W. Minor, XDISPLAYF (Purdue University, West Lafayette, IN, 1993)) or the SDMS software (A. J. Howard et al. *Meth. Enzymol.* 114:452-471 (1985)). Each data set was collected from a single crystal. Heavy atom soaks were 2 mM in mother liquor for 2 days. Initial electron density maps were based on three heavy atom derivatives using in-house data, then later were replaced with the synchrotron data. The EMTS difference Patterson map was solved by inspection, then used to calculate difference Fourier maps of the other derivatives. Lack of closure

refinement of the heavy atom parameters was performed using the Protein package (W. Steigemann, in Ph.D. Thesis (Technical University, Munich, 1974)). The MIR maps were much poorer than the overall figure of merit would suggest, and it was clear that the EMTS isomorphous differences dominated the phasing. The enhanced anomalous occupancy for the synchrotron data provided a partial solution to the problem. Note that the phasing power was reduced for the synchrotron data, but the figure of merit was unchanged. All experimental electron density maps were improved by solvent flattening using the program DM of the CCP4 (CCP4: *A Suite of Programs for Protein Crystallography* (SERC Daresbury Laboratory, Warrington WA4 4AD UK, 1979)) package assuming a solvent content of 38%. Phase combination was performed with PHASCO2 of the Protein package using a weight of 1.0 on the atomic model. Heavy atom parameters were subsequently improved by refinement against combined phases. Model building proceeded with FRODO and O (T. A. Jones et al. *Acta. Crystallogr. Sect. A* 47:110 (1991); T. A. Jones, in *Computational Crystallography* D. Sayre, Ed. (Oxford University Press, Oxford, 1982) pp. 303-317) and crystallographic refinement was performed with the TNT package (D. E. Tronrud et al. *Acta Cryst. A* 43:489-503 (1987)). Bond lengths and angles for the chromophore were estimated using CHEM3D (Cambridge Scientific Computing). Final refinement and model building was performed against the X4A selenomethione data set, using $(2F_o-F_c)$ electron density maps. The data beyond 1.9 Å resolution have not been used at this stage. The final model contains residues 2-229 as the terminal residues are not visible in the electron density map, and the side chains of several disordered surface residues have been omitted. Density is weak for residues 156-158 and coordinates for these residues are unreliable. This disordering is consistent with previous analyses showing that residues 1 and 233-238 are dispensible but that further truncations may prevent fluorescence (J. Dopf & T.M. Horiagon. *Gene* 173:39-43 (1996)). The atomic model has been deposited in the Protein Data Bank (access code 1EMA).

Table E

Diffraction Data Statistics

Crystal	Resolution (Å)	Total obs	Unique obs	Compl. (%)[a]	Compl. (shell)[b]	Rmerge (%)[c]	Riso (%)[d]
R-axix II							
Native	2.0	51907	13582	80	69	4.1	5.8
EMTS[e]	2.6	17727	6787	87	87	5.7	20.6
SeMet	2.3	44975	10292	92	88	10.2	9.3
Multiwire							
HGI4-Se	3.0	15380	4332	84	79	7.2	28.8
X4a							
SeMet	1.8	126078	19503	80	55	9.3	9.4
EMTS	2.3	57812	9204	82	66	7.2	26.3

Phasing Statistics

Derivative	Resolution (Å)	Number of sites	Phasing power[a]	Phasing Power(shell)	FOM[g]	FOM (shell)
In House						
EMTS	3.0	2	2.08	2.08	0.77	.072
SeMet	3.0	4	1.66	1.28	-	-
HGI4-Se	3.0	9	1.77	1.90	-	-
X4a						
EMTS	3.0	2	1.36	1.26	0.77	.072
SeMet	3.0	4	1.31	1.08	-	-

Atomic Model Statistics

Protein atoms	1790
Solvent atoms	94
Resol. range (Å)	20-1.9
Number of reflections (F > 0)	17676
Completeness	84.
R. factor[h]	0.175
Mean B-value (Å^2)	24.1

Deviations from ideality

Bond lengths (Å)	0.014
Bond angles (□)	1.9
Restrained B-values (Å^2)	4.3
Ramachandran outliers	0

Notes:

(a) Completeness is the ratio of observed reflections to theoretically possible expressed as a percentage.

(b) Shell indicates the highest resolution shell, typically 0.1-0.4 Å wide.

(c) Rmerge = □ |I - <I>| / □ I, where <I> is the mean of individual observations of intensities I.

(d) Riso = □ $|I_{DER} - I_{NAT}|$ / □ I_{NAT}

(e) Derivatives were EMTS=ethymercurithiosalicylate (residues modified Cys^{48} and Cys^{70}), SeMet=selenomethionine substituted protein (Met^{1} and Met^{233} could not be located); HgI_4-SeMet = double derivative HgI_4 on SeMet background.

(f) Phasing power = $<F_H>/<E>$ where $<F_H>$=r.m.s. heavy atom scattering and <E>=lack of closure.

(g) FOM, mean figure of merit

(h) Standard crystallographic R-factor, R = □ $||F_{obs}| - |F_{calc}||$ / □ $|F_{obs}|$

B. <u>Spectral properties of Thr^{203} ("T203") mutants compared to S65T</u>

The mutations F64L, V68L and S72A improve the folding of GFP at 37□ (B. P. Cormack et al. *Gene* 173:33 (1996)) but do not significantly shift the emission spectra.

TABLE F

Clone	Mutations	Excitation max.(nm)	Extinction coefficient ($10^3 M^{-1}cm^{-1}$)	Emission max.(nm)
S65T	S65T	489	39.2	511
5B	T203H/S65T	512	19.4	524
6C	T203Y/S65T	513	14.5	525
10B	T203Y/F64L/S65G/S72A	513	30.8	525
10C	T203Y/F65G/V68L/S72A	513	36.5	527
11	T203W/S65G/S72A	502	33.0	512

12H	T203Y/S65G/S72A	513	36.5	527
20A	T203Y/S65G/V68L/Q69K/S72A	515	46.0	527

The present invention provides novel long wavelength engineered fluorescent proteins. While specific examples have been provided, the above description is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification. The scope of the invention should, therefore, be determined not with reference to the above description, but instead should be determined with reference to the appended claims along with their full scope of equivalents.

All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent document were so individually denoted.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Regents of the University of California et al.

(ii) TITLE OF INVENTION: LONG WAVELENGTH MUTANT FLUORESCENT PROTEINS

(iii) NUMBER OF SEQUENCES: 4

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(F) ZIP: 92037

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vii) PRIOR APPLICATION DATA:
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(B) FILING DATE: 16-AUG-1996
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(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/706,408
(B) FILING DATE: 30-AUG-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Haile, Lisa A.
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(C) REFERENCE/DOCKET NUMBER: 07257/056WO1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 619/678-5070
(B) TELEFAX: 619/678-5099

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 716 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..714

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATG AGT AAA GGA GAA GAA CTT TTC ACT GCA GTT GTC CCA ATT CTT GTT          48
Met Ser Lys Gly Glu Glu Leu Phe Thr Ala Val Val Pro Ile Leu Val
 1               5                  10                  15

GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC AGT GGA GAG          96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30

GGT GAA GGT GAT GTA ACA TAC GGA AAA CTT ACC CTT AAA TTT ATT TGC         144
Gly Glu Gly Asp Val Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45

ACT ACT GGA AAA CTA CCT GTT CCA TGG CCA ACA CTT GTC ACT ACT TTC         192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60

TCT TAT GGT GTT CAA TGC TTT TCA AGA TAC CCA GAT CAT ATG AAA CGG         240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65                  70                  75                  80

CAT GAC TTT TTC AAG AGT GCC ATG CCC GAA GGT TAT GTA CAG CAA AGA         288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Gln Arg
                85                  90                  95

ACT ATA TTT TTC AAA GAT GAC GGG AAC TAC AAG ACA CGT GCT GAA GTC         336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110

AAG TTT GAA GGT GAT ACC CTT GTT AAT AGA ATC GAG TTA AAA GGT ATT         384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125

GAT TTT AAA GAA GAT GGA AAC ATT CTT GGA CAT AAA TTG GAA TAC AAC         432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

TAT AAC TCA CAC AAT GTA TAC ATC ATG GCA GAC AAA CAA AAG AAT GGA         480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

ATC AAA GTT AAC TTC AAA ATT AGA CAC AAC ATT GAA GAT GGA AGC GTT         528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175

CAA CTA GCA GAC TAT TAT CAA CAA AAT ACT CCA ATT CTC GAT GGC CCT         576
Gln Leu Ala Asp Tyr Tyr Gln Gln Asn Thr Pro Ile Leu Asp Gly Pro
            180                 185                 190

GTC CTT TTA CCA GAC AAC CAT TAC CTG TCC ACA CAA TCT GCC CTT TCG         624
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

AAA GAT CCC AAC GAA AAG AGA GAC CAC ATG GTC CTT CTT GAG TTT GTA         672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

ACA GCT GCT GGG ATT ACA CAT GGC ATG GAT GAA CTA TAC AAA                 714
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235

TA                                                                     716
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 238 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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 1               5                  10                  15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30

Gly Glu Gly Asp Val Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65                  70                  75                  80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Gln Arg
                85                  90                  95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175

Gln Leu Ala Asp Tyr Tyr Gln Gln Asn Thr Pro Ile Leu Asp Gly Pro
            180                 185                 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 720 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..720

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG GTG GTG CCC ATC CTG          48
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
    240                 245                 250

GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC GGC          96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
255                 260                 265                 270

GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC         144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
                275                 280                 285

TGC ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC         192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
            290                 295                 300

TTC GGC TAC GGC GTG CAG TGC TTC GCC CGC TAC CCC GAC CAC ATG AAG         240
Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
        305                 310                 315

CAG CAG GAC TTC TTC AAG TCC GCC ATG CCC GAA GGC TAC GTC CAG GAG         288
Gln Gln Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
    320                 325                 330

CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG         336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
335                 340                 345                 350

GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC         384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
                355                 360                 365

ATC GAC TTC AAG GAC GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC         432
Ile Asp Phe Lys Asp Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
            370                 375                 380

AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC         480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
        385                 390                 395

GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC         528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
    400                 405                 410

GTG CAG CCC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC         576
Val Gln Pro Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
415                 420                 425                 430

CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC TAC CAG TCC GCC CTG         624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
                435                 440                 445

AGC AAA GAC CCC AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG GAG TTC         672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
            450                 455                 460

GTG ACC GCC GCC GGG ATC ACT CAC GGC ATG GAC GAG CTG TAC AAG TAA         720
Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  *
        465                 470                 475
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 240 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1 5 10 15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
65 70 75 80

Gln Gln Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

Ile Asp Phe Lys Asp Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145 150 155 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

Val Gln Pro Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
195 200 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys *
225 230 235 240

WHAT IS CLAIMED IS:

1. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

2. The nucleic acid molecule of claim 1 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

3. The nucleic acid molecule of claim 1 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

4. The nucleic acid molecule of claim 1 or 2 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

5. The nucleic acid molecule of claim 1 or 2 wherein the amino acid sequence further comprises a mutation from Table A.

6. The nucleic acid molecule of claim 1 or 2 wherein the amino acid sequence further comprises a folding mutation.

7. The nucleic acid molecule of any of claims 1-3 wherein the nucleotide sequence encoding the protein differs from the nucleotide sequence of SEQ ID NO:1 by the substitution of at least one codon by a preferred mammalian codon.

8. The nucleic acid molecule of any of claims 1-3 encoding a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

9. An expression vector comprising expression control sequences operatively linked to a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

10. The expression vector of claim 9 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

11. The expression vector of claim 9 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y, S65G/S72A/T203Y; or S65G/S72A/T203W.

12. The expression vector of claim 10 or 11 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

13. The expression vector of claim 10 or 11 wherein the amino acid sequence further comprises a mutation from Table A.

14. The expression vector of claim 9 or 10 wherein the amino acid sequence further comprises a folding mutation.

15. The expression vector of any of claims 9-11 wherein the nucleotide sequence encoding the protein differs from the nucleotide sequence of SEQ ID NO:1 by the substitution of at least one codon by a preferred mammalian codon.

16. The expression vector of any of claims 9-11 encoding a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

17. A recombinant host cell comprising an expression vector that comprises expression control sequences operatively linked to a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

18. The recombinant host cell of claim 17 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

19. The recombinant host cell of claim 17 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

20. The recombinant host cell of claim 17 or 18 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

21. The recombinant host cell of claim 17 or 18 wherein the amino acid sequence further comprises a mutation from Table A.

22. The recombinant host cell of claim 17 or 18 wherein the amino acid sequence further comprises a folding mutation.

23. The recombinant host cell of any of claims 17-19 wherein the nucleotide sequence encoding the protein differs from the nucleotide sequence of SEQ ID NO:1 by the substitution of at least one codon by a preferred mammalian codon.

24. The recombinant host cell of any of claims 17-19 encoding a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

25. The recombinant host cell of any of claims 17-19 which is a prokaryotic cell.

26. The recombinant host cell of any of claims 17-19 which is a eukaryotic cell.

27. A functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

28. The protein of claim 27 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

29. The protein of claim 27 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

30. The protein of claim 27 or 28 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

31. The protein of claim 27 or 28 wherein the amino acid sequence further comprises a folding mutation.

32. The protein of any of claims 27-29 which is a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

33. A fluorescently labelled antibody comprising an antibody coupled to a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

34. The fluorescently labelled antibody of claim 33 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

35. The fluorescently labelled antibody of claim 33 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

36. The fluorescently labelled antibody of claim 33 or 34 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

37. The fluorescently labelled antibody of any of claims 33-35 which is a fusion protein wherein the fusion protein comprises the antibody fused to the functional engineered fluorescent protein.

38. A nucleic acid molecule comprising a nucleotide sequence encoding an antibody fused to a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

39. The nucleic acid molecule of claim 38 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

40. The nucleic acid molecule of claim 38 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

41. The nucleic acid molecule of claim 38 or 39 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

42. A fluorescently labelled nucleic acid probe comprising a nucleic acid probe coupled to a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

43. The fluorescently labelled nucleic acid probe of claim 42 wherein the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

44. The fluorescently labelled nucleic acid probe of claim 42 wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

45. The nucleic acid molecule of claim 42 or 43 wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

46. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

47. The nucleic acid molecule of claim 46 wherein the amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,

V61X, wherein X is selected from F, Y, H and C,

T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,

V68X, wherein X is selected from F, Y and H,

Q69X, wherein X is selected from K, R, E and G,

Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,

H148X, wherein X is selected from F, Y, N, K, Q and R,

V150X, wherein X is selected from F, Y and H,

F165X, wherein X is selected from H, Q, W and Y,

I167X, wherein X is selected from F, Y and H,

Q183X, wherein X is selected from H, Y, E and K,

N185X, wherein X is selected from D, E, H, K and Q,

L220X, wherein X is selected from H, N, Q and T,

E222X, wherein X is selected from N and Q or

V224X, wherein X is selected from H, N, Q, T, F, W and Y.

48. An expression vector comprising expression control sequences operatively linked to a nucleic acid molecule of comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

49. The expression vector of claim 48 wherein the amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,

V61X, wherein X is selected from F, Y, H and C,

T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,

V68X, wherein X is selected from F, Y and H,

Q69X, wherein X is selected from K, R, E and G,

Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,

H148X, wherein X is selected from F, Y, N, K, Q and R,

V150X, wherein X is selected from F, Y and H,

F165X, wherein X is selected from H, Q, W and Y,

I167X, wherein X is selected from F, Y and H,

Q183X, wherein X is selected from H, Y, E and K,

N185X, wherein X is selected from D, E, H, K and Q,

L220X, wherein X is selected from H, N, Q and T,

E222X, wherein X is selected from N and Q or

V224X, wherein X is selected from H, N, Q, T, F, W and Y.

50. A recombinant host cell comprising an expression vector that comprises expression control sequences operatively linked to a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

51. The recombinant host cell of claim 50 wherein the amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,

V61X, wherein X is selected from F, Y, H and C,

T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,

V68X, wherein X is selected from F, Y and H,

Q69X, wherein X is selected from K, R, E and G,

Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,

H148X, wherein X is selected from F, Y, N, K, Q and R,

V150X, wherein X is selected from F, Y and H,

F165X, wherein X is selected from H, Q, W and Y,

I167X, wherein X is selected from F, Y and H,

Q183X, wherein X is selected from H, Y, E and K,

N185X, wherein X is selected from D, E, H, K and Q,

L220X, wherein X is selected from H, N, Q and T,

E222X, wherein X is selected from N and Q or

V224X, wherein X is selected from H, N, Q, T, F, W and Y.

52. A functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

53. The functional engineered fluorescent protein of claim 52 wherein the amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,

V61X, wherein X is selected from F, Y, H and C,

T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,

V68X, wherein X is selected from F, Y and H,

Q69X, wherein X is selected from K, R, E and G,

Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,

H148X, wherein X is selected from F, Y, N, K, Q and R,

V150X, wherein X is selected from F, Y and H,

F165X, wherein X is selected from H, Q, W and Y,

I167X, wherein X is selected from F, Y and H,

Q183X, wherein X is selected from H, Y, E and K,

N185X, wherein X is selected from D, E, H, K and Q,

L220X, wherein X is selected from H, N, Q and T,

E222X, wherein X is selected from N and Q or

V224X, wherein X is selected from H, N, Q, T, F, W and Y.

54. A fluorescently labelled antibody comprising an antibody coupled to a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

55. The antibody of claim 54 wherein the amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,

V61X, wherein X is selected from F, Y, H and C,

T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,

V68X, wherein X is selected from F, Y and H,

Q69X, wherein X is selected from K, R, E and G,

Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,
H148X, wherein X is selected from F, Y, N, K, Q and R,
V150X, wherein X is selected from F, Y and H,
F165X, wherein X is selected from H, Q, W and Y,
I167X, wherein X is selected from F, Y and H,
Q183X, wherein X is selected from H, Y, E and K,
N185X, wherein X is selected from D, E, H, K and Q,
L220X, wherein X is selected from H, N, Q and T,
E222X, wherein X is selected from N and Q or
V224X, wherein X is selected from H, N, Q, T, F, W and Y.

56. A nucleic acid molecule comprising a nucleotide sequence encoding an antibody fused to a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

57. The nucleic acid molecule of claim 56 wherein the amino acid substitution is:
L42X, wherein X is selected from C, F, H, W and Y,
V61X, wherein X is selected from F, Y, H and C,
T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,
V68X, wherein X is selected from F, Y and H,
Q69X, wherein X is selected from K, R, E and G,
Q94X, wherein X is selected from D, E, H, K and N,
N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,

H148X, wherein X is selected from F, Y, N, K, Q and R,

V150X, wherein X is selected from F, Y and H,

F165X, wherein X is selected from H, Q, W and Y,

I167X, wherein X is selected from F, Y and H,

Q183X, wherein X is selected from H, Y, E and K,

N185X, wherein X is selected from D, E, H, K and Q,

L220X, wherein X is selected from H, N, Q and T,

E222X, wherein X is selected from N and Q or

V224X, wherein X is selected from H, N, Q, T, F, W and Y.

58. A fluorescently labelled nucleic acid probe comprising a nucleic acid probe coupled to a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

59. The probe of claim 58 wherein the amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,

V61X, wherein X is selected from F, Y, H and C,

T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,

V68X, wherein X is selected from F, Y and H,

Q69X, wherein X is selected from K, R, E and G,

Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,

H148X, wherein X is selected from F, Y, N, K, Q and R,

V150X, wherein X is selected from F, Y and H,

F165X, wherein X is selected from H, Q, W and Y,

I167X, wherein X is selected from F, Y and H,

Q183X, wherein X is selected from H, Y, E and K,

N185X, wherein X is selected from D, E, H, K and Q,

L220X, wherein X is selected from H, N, Q and T,

E222X, wherein X is selected from N and Q or

V224X, wherein X is selected from H, N, Q, T, F, W and Y.

60. A method for determining whether a mixture contains a target comprising:

contacting the mixture with a fluorescently labelled probe comprising a probe and a functional engineered fluorescent protein of claim 27 or claim 52; and

determining whether the target has bound to the probe.

61. The method of any of claim 60 the target is bound to a solid matrix.

62. A method for engineering a functional engineered fluorescent protein having a fluorescent property different than *Aequorea* green fluorescent protein, comprising substituting an amino acid that is located no more than 0.5 nm from any atom in the chromophore of an *Aequorea*-related green fluorescent protein with another amino acid; whereby the substitution alters a fluorescent property of the protein.

63. The method of claim 62 wherein the amino acid substitution alters the electronic environment of the chromophore.

64. A method for engineering a functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein comprising substituting amino acids in a loop domain of an *Aequorea*-related green fluorescent protein with amino acids so as to create a consensus sequence for phosphorylation or for proteolysis.

65. A method for producing fluorescence resonance energy transfer comprising:

providing a donor molecule comprising a functional engineered fluorescent protein of claim 27 or claim 52;

providing an appropriate acceptor molecule for the fluorescent protein; and

bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy transfer.

66. A method for producing fluorescence resonance energy transfer comprising:

providing an acceptor molecule comprising a functional engineered fluorescent protein of claim 27 or claim 52;

providing an appropriate donor molecule for the fluorescent protein; and

bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy transfer.

67. The method of claim 66 wherein the donor molecule is a engineered fluorescent protein whose amino acid sequence comprises the substitution T203I and the acceptor molecule is a nutant fluorescent protein whose amino acid sequence comprises the substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than *Aequorea* green fluorescent protein.

68. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 nm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

69. An expression vector comprising expression control sequences operatively linked to a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 nm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

70. A functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 nm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

71. A crystal of a protein comprising a fluorescent protein with an amino acid sequence substantially identical to SEQ ID NO: 2, wherein said crystal diffracts with at least a 2.0 to 3.0 angstrom resolution.

72. The crystal of claim 71, wherein the fluorescent protein has at least 200 amino acids, a completeness value of at least 80% and has a crystal stability within 0.5% of its unit cell dimensions.

73. The crystal of claim 71, wherein the amino acid sequence comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I.

74. The crystal of claim 71, wherein said crystal has the following unit cell dimensions in angstroms: a = 51.8, b= 62.8 and c= 70.7 with a space group of P2 2 2 and an □ angle of 90.00□, a □ angle of 90.00□ and a □ angle of 90.00□ and the crystal has a diffraction limit where 90% or greater of the potential reflections can be used to determine the coordinates of the atoms.

75. A computational method of designing a fluoresent protein comprising:

determining from a three dimensional model of a crystallized fluorescent protein comprising a fluorescent protein with a bound ligand, at least one interacting amino acid of the fluorescent protein that interacts with at least one first chemical moiety of the ligand, and

selecting at least one chemical modification of the first chemical moiety to produce a second chemical moiety with a structure to either decrease or increase an interaction between the interacting amino acid and the second chemical moiety compared to the interaction between the interacting amino acid and the first chemical moiety.

76. The computational method of claim 75, further comprising generating the three dimensional model of the crystallized protein comprising a fluorescent protein with an amino acid sequence substantially identical to SEQ ID NO:2.

77. The computational method of claim 75, wherein the selecting selects the first chemical moiety that interacts with at least one of the amino acids listed in Figs. 5-1 to 5-28.

78. The computational method of claim 75, wherein the chemical modification enhances hydrogen bonding interaction, charge interaction, hydrophobic interaction, Van Der Waals interaction or dipole interaction between the second chemical moiety and the interacting amino acid compared to the first chemical moiety and the interacting amino acid.

79. A computational method of modeling the three dimensional structure of a fluorescent protein comprising determining a three dimensional relationship between at least two atoms listed in the atomic coordinates of Figs. 5-1 to 5-28.

80. The computational method of claim 79, wherein the determining comprises determining the three dimensional structure of a fluorescent protein with an amino acid sequence at least 80% identical to SEQ ID NO:2.

81. The computational method of claim 79, wherein the determining comprises determining the three dimensional structure of a fluorescent protein with an amino acid sequence at least 95% identical to SEQ ID NO:2.

82. The computational method of claim 79, wherein the determining comprises determining the three dimensional relationship of at least 1500 atoms listed in Figs. 5-1 to 5-28.

83. A device comprising a storage device and, stored in the device, at least 10 atomic coordinates selected from the atomic coordinates listed in Figs. 5-1 to 5-28.

84. The device of claim 83, wherein the storage device is a computer readable device that stores code that receives as input the atomic coordinates.

85. The device of claim 84, wherein computer readable device is a floppy disk or a hard drive.

86. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at Q69, wherein said functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

87. The nucleic acid molecule of claim 86, wherein said substitution at Q69 is selected from the group of K, R, E and G.

88. The nucleic acid molecule of claim 86, wherein said amino acid sequence further comprises a function mutation at S65.

89. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at E222, but not including E222G, wherein said functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

90. The nucleic acid molecule of claim 89, wherein said substitution at E222 is selected from the group of N and Q.

91. The nucleic acid molecule of claim 89, wherein said amino acid sequence further comprises a function mutation at F64.

92. A nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at Y145, wherein said functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.

93. The nucleic acid molecule of claim 92, wherein said substitution at Y145 is selected from the group of W, C, F, L, E, H, K and Q .

94. The nucleic acid molecule of claim 92, wherein said amino acid sequence further comprises a function mutation at Y66.

95. A method of identifying a test chemical, comprising:

contacting a test chemical a sample containing a biological entity labeled with a functional, engineered fluorescent protein or a polynucleotide encoding said functional, engineered fluorescent protein, and

detecting fluorescence of said functional engineered fluorescent protein.

96. The method of claim 95, wherein said fluorescence in the presence of a test chemical is greater than in the absence of said test chemical.

97. The method of claim 96, wherein said polynucleotide encoding said functional, engineered fluorescent protein is operatively linked to a genomic polynucleotide.

98. The method of claim 95, wherein said functional, engineered fluorescent protein is fused to second functional protein.

99. The method of claim 96, wherein said polynucleotide encoding said functional, engineered fluorescent protein is operatively linked to a response element.

100. The method of claim 96, wherein said polynucleotide encoding said functional, engineered fluorescent protein is operatively linked to a response element in a mammalian cell.

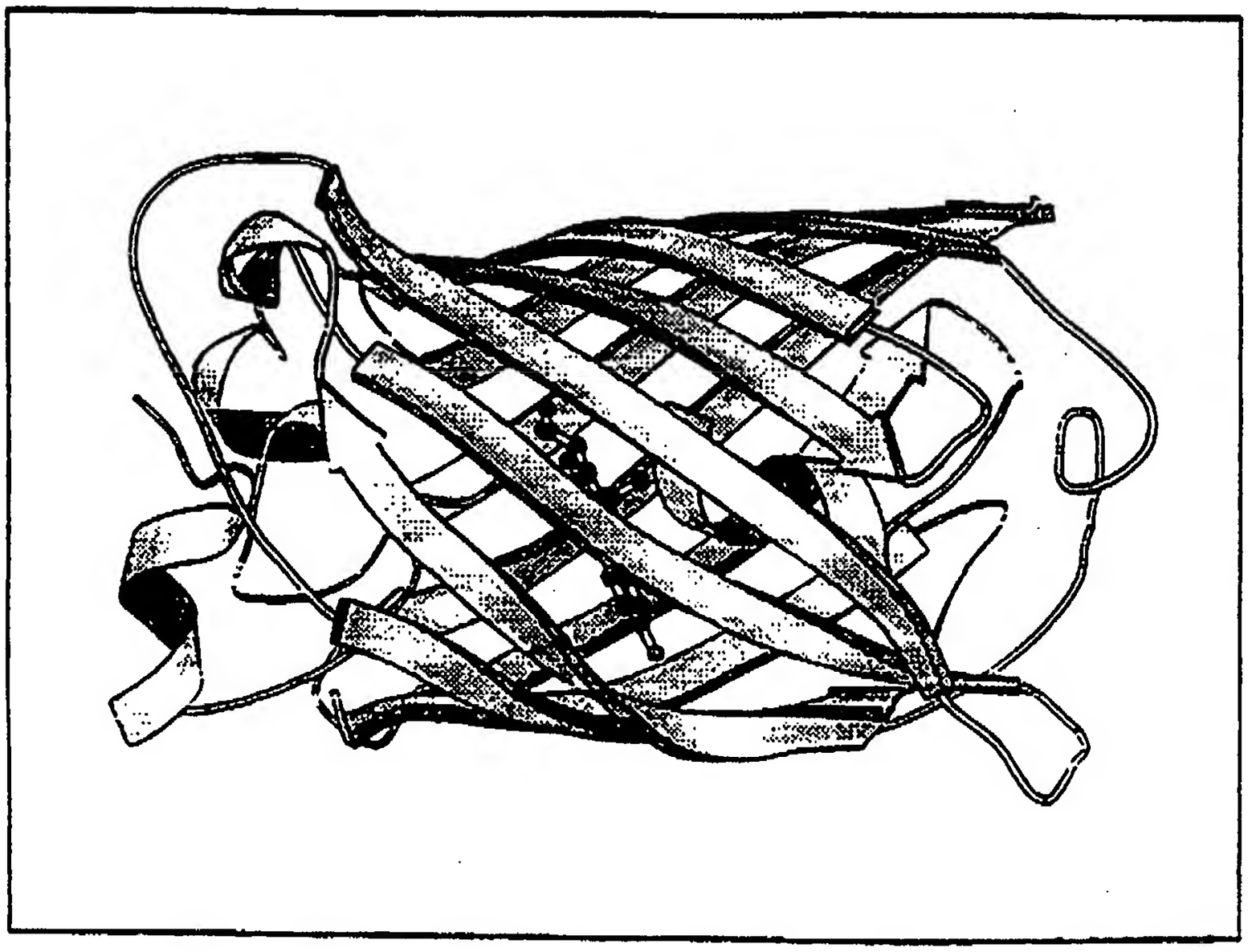

Figure 1a

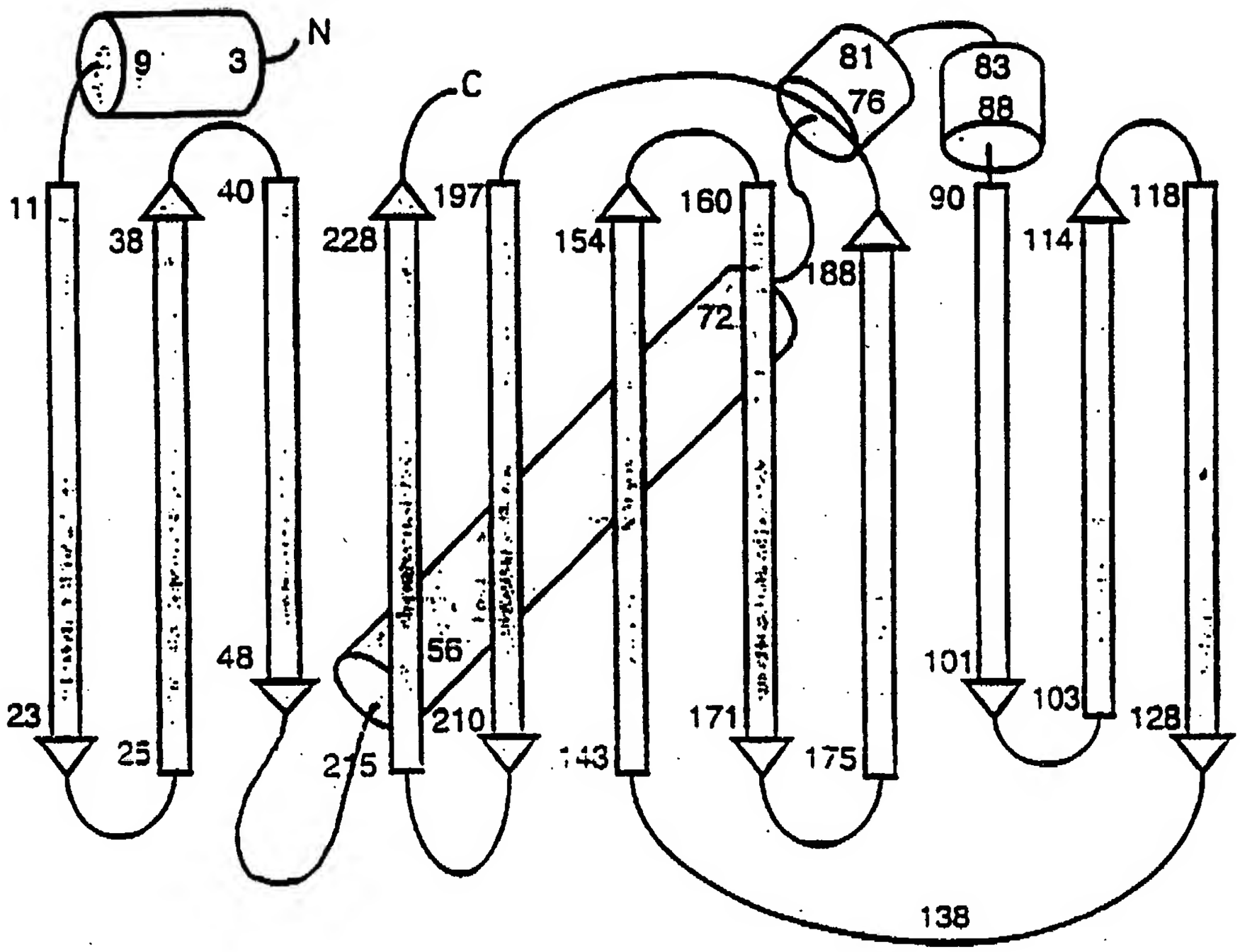
N
9
3
C
81
76
83
88
11
38
40
228
197
154
160
188
72
90
114
118
48
56
101
103
23
25
210
215
171
175
128
143
138

Figure 1b

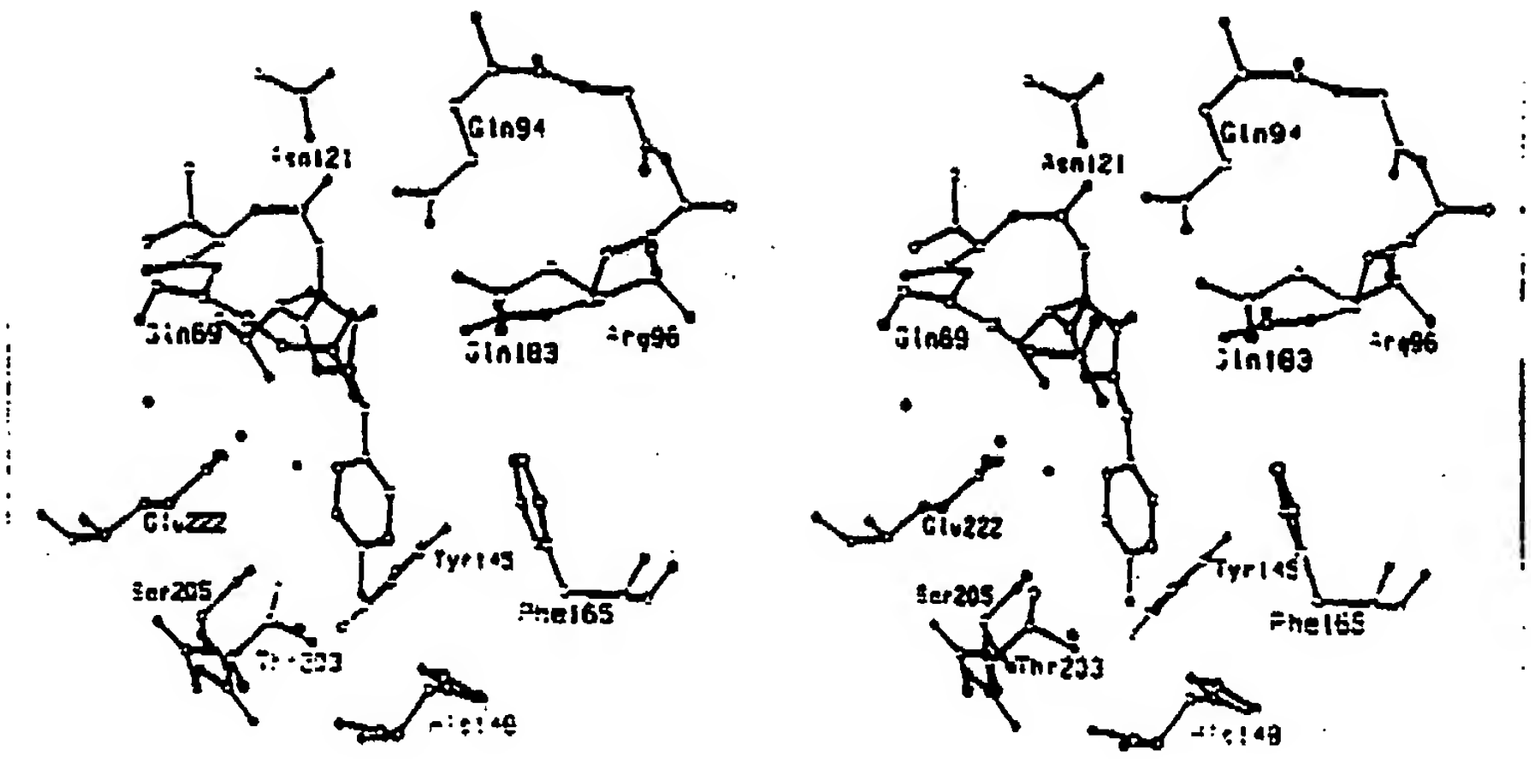
Asn121
Gln94
Gln69
Gln183
Arg96
Glu222
Tyr145
Ser205
Phe165
Thr203
Asn121
Gln94
Gln69
Gln183
Arg96
Glu222
Tyr145
Ser205
Phe165
Thr203

Figure 2a

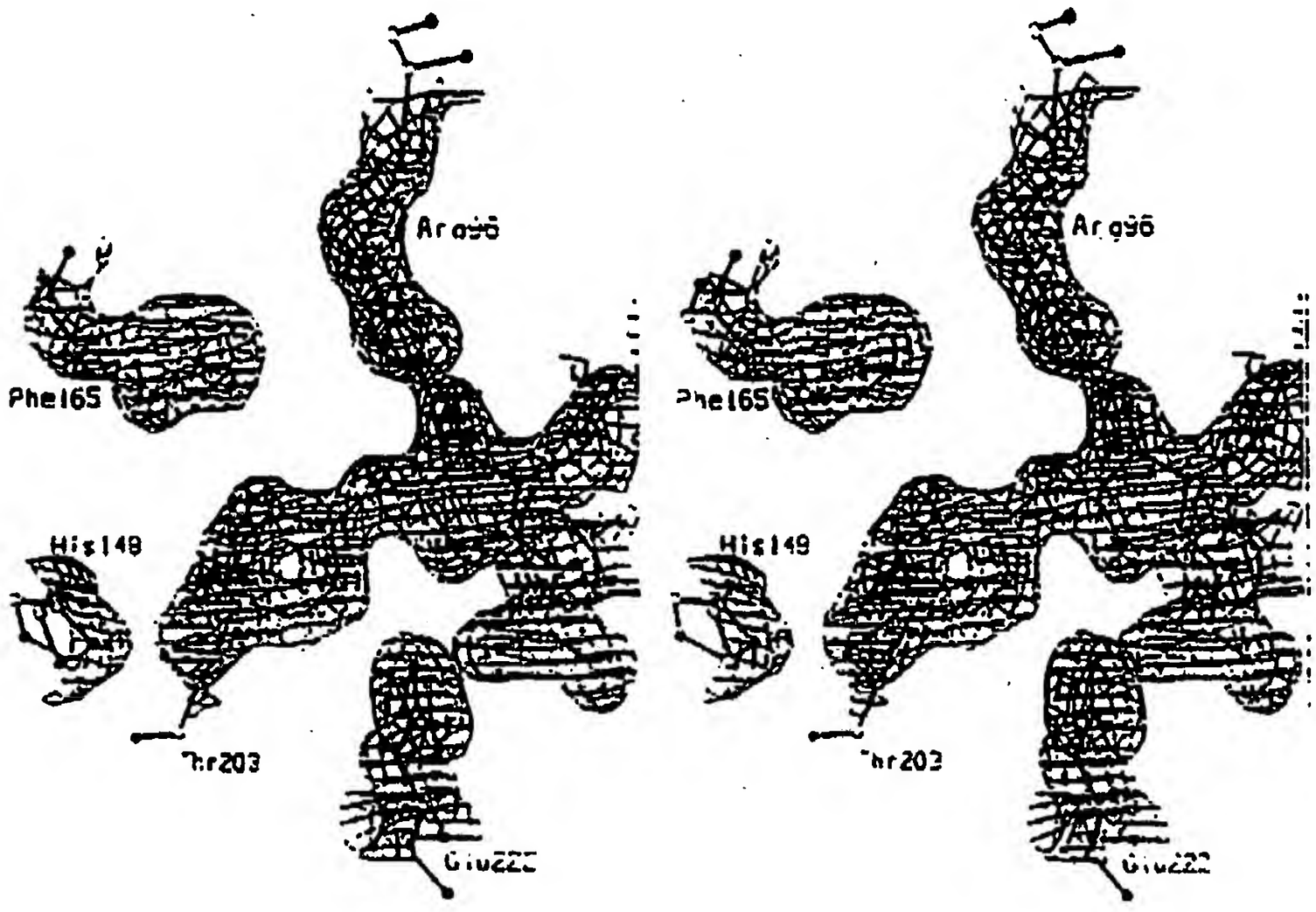
Arg96
Phe165
His149
Thr203
Glu222
Arg96
Phe165
His149
Thr203
Glu222

Figure 2b

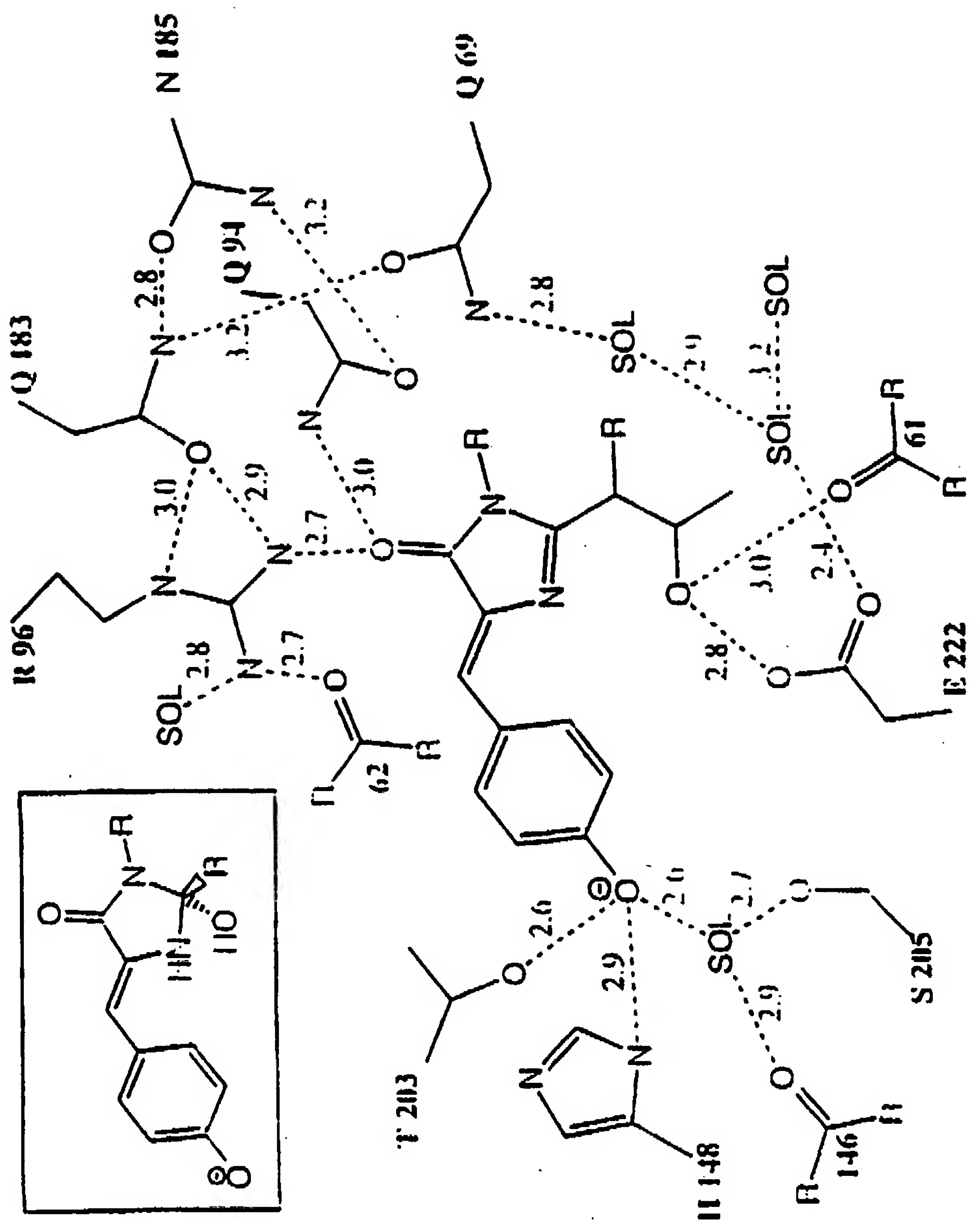

Figure 2c

(xi) SEQUENCE DESCRIPTION:

SEQ ID NO:1: ATG AGT AAA GGA GAA GAA CTT TTC ACT GGA GTT GTC CCA ATT CTT GTT 48
SEQ ID NO:2: Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1 5 10 15

GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC AGT GGA GAG 96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
20 25 30

GGT GAA GGT GAT GCA ACA TAC GGA AAA CTT ACC CTT AAA TTT ATT TGC 144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
35 40 45

ACT ACT GGA AAA CTA CCT GTT CCA TGG CCA ACA CTT GTC ACT ACT TTC 192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
50 55 60

TCT TAT GGT GTT CAA TGC TTT TCA AGA TAC CCA GAT CAT ATG AAA CGG 240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65 70 75 80

CAT GAC TTT TTC AAG AGT GCC ATG CCC GAA GGT TAT GTA CAG GAA AGA 288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95

ACT ATA TTT TTC AAA GAT GAC GGG AAC TAC AAG ACA CGT GCT GAA GTC 336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110

AAG TTT GAA GGT GAT ACC CTT GTT AAT AGA ATC GAG TTA AAA GGT ATT 384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

GAT TTT AAA GAA GAT GGA AAC ATT CTT GGA CAC AAA TTG GAA TAC AAC 432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
130 135 140

TAT AAC TCA CAC AAT GTA TAC ATC ATG GCA GAC AAA CAA AAG AAT GGA 480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145 150 155 160

ATC AAA GTT AAC TTC AAA ATT AGA CAC AAC ATT GAA GAT GGA AGC GTT 528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
165 170 175

CAA CTA GCA GAC CAT TAT CAA CAA AAT ACT CCA ATT GGC GAT GGC CCT 576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190

GTC CTT TTA CCA GAC AAC CAT TAC CTG TCC ACA CAA TCT GCC CTT TCG 624
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205

AAA GAT CCC AAC GAA AAG AGA GAC CAC ATG GTC CTT CTT GAG TTT GTA 672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220

ACA GCT GCT GGG ATT ACA CAT GGC ATG GAT GAA CTA TAC AAA TA 717
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

Figure 3

Table 3653. 5'CA humanized codon usage. With an additional amino acid after the start met to provide optimal Kozak sequence

```
                 9         18        27        36        45        54
ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGC GTG GTG CCC ATC CTG GTC GAG
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu

         63        72        81        90        99       108
CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC GGC GAG GGC GAG GGC
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly

        117       126       135       144       153       162
GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC ACC ACC GGC AAG CTG
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu

        171       180       189       198       207       216
CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC TTC GGC TAC GGC GTG CAG TGC TTC
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Gly Tyr Gly Val Gln Cys Phe

        225       234       243       252       261       270
GCC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC GCC ATG CCC
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro

        279       288       297       306       315       324
GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys

        333       342       351       360       369       378
ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu

        387       396       405       414       423       432
AAG GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr

        441       450       459       468       477       486
AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile

        495       504       513       522       531       540
AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG CTC GCC
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala

        549       558       567       576       585       594
GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC GAC
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp

        603       612       621       630       639       648
AAC CAC TAC CTG AGC TAC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg

        657       666       675       684       693       702
GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CAC GGC ATG
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met

                711
GAC GAG CTG TAC AAG TAA )'
... ... ... ... ...
Asp Glu Leu Tyr Lys ***
```

Figure 4

FIG 5-1

```
CRYST1   51.767   62.845   70.666  90.00  90.00  90.00
ORIGX1      1.000000  0.000000  0.000000        0.00000
ORIGX2      0.000000  1.000000  0.000000        0.00000
ORIGX3      0.000000  0.000000  1.000000        0.00000
SCALE1      0.019317  0.000000  0.000000        0.00000
SCALE2      0.000000  0.015912  0.000000        0.00000
SCALE3      0.000000  0.000000  0.014151        0.00000
ATOM      1  N   SER     2      28.888   9.409  52.301  1.00 85.05
ATOM      2  CA  SER     2      27.638  10.125  52.516  1.00 80.05
ATOM      3  C   SER     2      26.499   9.639  51.644  1.00 85.36
ATOM      4  O   SER     2      26.606   8.656  50.915  1.00 84.56
ATOM      5  CB  SER     2      27.783  11.635  52.378  1.00 70.97
ATOM      6  OG  SER     2      27.690  12.033  51.012  1.00 44.08
ATOM      7  N   LYS     3      25.418  10.403  51.731  1.00 87.71
ATOM      8  CA  LYS     3      24.141  10.191  51.036  1.00 87.15
ATOM      9  C   LYS     3      24.214  10.266  49.497  1.00 76.86
ATOM     10  O   LYS     3      24.107   9.258  48.774  1.00 78.27
ATOM     11  CB  LYS     3      23.127  11.240  51.521  1.00 89.44
ATOM     12  CG  LYS     3      21.768  10.697  51.949  1.00 75.06
ATOM     13  CD  LYS     3      20.681  11.781  51.987  1.00 76.58
ATOM     14  CE  LYS     3      20.711  12.655  53.243  1.00 68.55
ATOM     15  NZ  LYS     3      20.816  14.103  52.953  1.00 46.24
ATOM     16  N   GLY     4      24.318  11.495  49.015  1.00 53.62
ATOM     17  CA  GLY     4      24.297  11.798  47.605  1.00 45.97
ATOM     18  C   GLY     4      25.425  11.206  46.796  1.00 31.90
ATOM     19  O   GLY     4      25.234  10.923  45.619  1.00 33.63
ATOM     20  N   GLU     5      26.606  11.082  47.420  1.00 32.54
ATOM     21  CA  GLU     5      27.821  10.598  46.726  1.00 32.57
ATOM     22  C   GLU     5      27.523   9.590  45.616  1.00 28.40
ATOM     23  O   GLU     5      27.850   9.803  44.444  1.00 26.12
ATOM     24  CB  GLU     5      28.873  10.053  47.718  1.00 38.53
ATOM     25  CG  GLU     5      30.337  10.461  47.425  1.00 41.36
ATOM     26  CD  GLU     5      31.311   9.584  48.170  1.00 90.82
ATOM     27  OE1 GLU     5      31.508   9.677  49.381  1.00 74.80
ATOM     28  OE2 GLU     5      31.839   8.653  47.403  1.00100.00
ATOM     29  N   GLU     6      26.883   9.499  46.017  1.00 28.57
ATOM     30  CA  GLU     6      26.479   7.410  45.150  1.00 31.50
ATOM     31  C   GLU     6      25.561   7.837  43.979  1.00 31.10
ATOM     32  O   GLU     6      25.479   7.142  42.955  1.00 30.96
ATOM     33  CB  GLU     6      25.780   6.330  45.992  1.00 35.64
ATOM     34  CG  GLU     6      25.260   6.893  47.338  1.00 55.53
ATOM     35  N   LEU     7      24.864   9.966  44.138  1.00 22.26
ATOM     36  CA  LEU     7      23.954   9.456  43.089  1.00 21.61
ATOM     37  C   LEU     7      24.693  10.061  41.917  1.00 16.90
ATOM     38  O   LEU     7      24.152  10.250  40.836  1.00 18.38
ATOM     39  CB  LEU     7      23.050  10.548  43.665  1.00 22.41
ATOM     40  CG  LEU     7      21.672  10.058  44.098  1.00 32.84
ATOM     41  CD1 LEU     7      21.597   8.536  44.074  1.00 31.64
ATOM     42  CD2 LEU     7      21.332  10.591  45.485  1.00 33.14
ATOM     43  N   PHE     8      25.944  10.407  42.157  1.00 20.75
ATOM     44  CA  PHE     8      26.740  11.132  41.169  1.00 21.64
ATOM     45  C   PHE     8      27.818  10.333  40.427  1.00 30.58
ATOM     46  O   PHE     8      28.590  10.856  39.600  1.00 30.05
ATOM     47  CB  PHE     8      27.309  12.376  41.820  1.00 16.95
ATOM     48  CG  PHE     8      26.222  13.355  42.163  1.00 13.29
ATOM     49  CD1 PHE     8      25.672  13.378  43.447  1.00 17.27
ATOM     50  CD2 PHE     8      25.725  14.227  41.189  1.00 13.12
ATOM     51  CE1 PHE     8      24.661  14.290  43.772  1.00 15.14
ATOM     52  CE2 PHE     8      24.712  15.137  41.499  1.00 13.19
ATOM     53  CZ  PHE     8      24.192  15.170  42.794  1.00  5.69
ATOM     54  N   THR     9      27.798   8.074  40.699  1.00 27.35
ATOM     55  CA  THR     9      28.704   8.122  40.175  1.00 34.93
ATOM     56  C   THR     9      28.709   7.992  38.636  1.00 45.22
ATOM     57  O   THR     9      29.642   7.452  38.062  1.00 50.55
ATOM     58  CB  THR     9      28.447   9.795  40.892  1.00 44.60
ATOM     59  OG1 THR     9      29.639   9.331  41.537  1.00 40.40
ATOM     60  CG2 THR     9      27.301   8.779  39.959  1.00 39.76
```

FIG 5-2

```
ATOM     61  N   GLY  10      27.690   8.510  37.956  1.00 30.53
ATOM     62  CA  GLY  10      27.689   8.458  36.507  1.00 23.21
ATOM     63  C   GLY  10      27.144   9.746  35.914  1.00 16.55
ATOM     64  O   GLY  10      27.011  10.729  36.617  1.00 25.70
ATOM     65  N   VAL  11      26.835   9.719  34.629  1.00 16.39
ATOM     66  CA  VAL  11      26.209  10.863  33.971  1.00 22.28
ATOM     67  C   VAL  11      24.758  11.020  34.479  1.00 29.60
ATOM     68  O   VAL  11      23.972  10.062  34.456  1.00 20.43
ATOM     69  CB  VAL  11      26.173  10.664  32.467  1.00 30.87
ATOM     70  CG1 VAL  11      25.912  11.980  31.734  1.00 31.75
ATOM     71  CG2 VAL  11      27.480  10.048  32.015  1.00 33.85
ATOM     72  N   VAL  12      24.417  12.227  34.931  1.00 20.12
ATOM     73  CA  VAL  12      23.080  12.561  35.433  1.00 12.88
ATOM     74  C   VAL  12      22.407  13.624  34.516  1.00 14.37
ATOM     75  O   VAL  12      23.007  14.639  34.179  1.00 13.42
ATOM     76  CB  VAL  12      23.270  13.077  36.839  1.00 15.01
ATOM     77  CG1 VAL  12      22.000  13.662  37.422  1.00 17.57
ATOM     78  CG2 VAL  12      23.781  11.936  37.728  1.00 16.55
ATOM     79  N   PRO  13      21.180  13.382  34.066  1.00 14.72
ATOM     80  CA  PRO  13      20.493  14.382  33.265  1.00 10.76
ATOM     81  C   PRO  13      20.116  15.589  34.141  1.00  7.65
ATOM     82  O   PRO  13      19.797  15.468  35.337  1.00 15.14
ATOM     83  CB  PRO  13      19.225  13.707  32.745  1.00 17.36
ATOM     84  CG  PRO  13      19.043  12.422  33.550  1.00 19.69
ATOM     85  CD  PRO  13      20.315  12.195  34.340  1.00 15.41
ATOM     86  N   ILE  14      20.196  16.766  33.557  1.00 14.91
ATOM     87  CA  ILE  14      19.893  17.991  34.266  1.00 12.93
ATOM     88  C   ILE  14      18.768  18.760  33.596  1.00 12.08
ATOM     89  O   ILE  14      18.724  18.878  32.399  1.00 11.04
ATOM     90  CB  ILE  14      21.109  18.905  34.325  1.00 16.54
ATOM     91  CG1 ILE  14      22.271  18.169  35.015  1.00 18.08
ATOM     92  CG2 ILE  14      20.783  20.207  35.084  1.00 11.56
ATOM     93  CD1 ILE  14      23.642  18.836  34.738  1.00 16.15
ATOM     94  N   LEU  15      17.899  19.307  34.421  1.00 13.85
ATOM     95  CA  LEU  15      16.811  20.136  33.955  1.00 14.82
ATOM     96  C   LEU  15      16.915  21.474  34.685  1.00  3.62
ATOM     97  O   LEU  15      17.080  21.509  35.901  1.00 10.00
ATOM     98  CB  LEU  15      15.462  19.450  34.285  1.00 21.25
ATOM     99  CG  LEU  15      14.412  19.541  33.199  1.00 40.50
ATOM    100  CD1 LEU  15      13.279  20.440  33.679  1.00 46.97
ATOM    101  CD2 LEU  15      15.008  20.098  31.913  1.00 49.22
ATOM    102  N   VAL  16      16.885  22.556  33.919  1.00 10.56
ATOM    103  CA  VAL  16      16.964  23.905  34.479  1.00 10.23
ATOM    104  C   VAL  16      15.716  24.727  34.063  1.00  9.47
ATOM    105  O   VAL  16      15.347  24.748  32.904  1.00 16.72
ATOM    106  CB  VAL  16      18.273  24.668  34.098  1.00 12.85
ATOM    107  CG1 VAL  16      18.226  26.075  34.691  1.00 12.58
ATOM    108  CG2 VAL  16      19.520  23.945  34.628  1.00 14.24
ATOM    109  N   GLU  17      15.059  25.317  35.060  1.00 14.43
ATOM    110  CA  GLU  17      13.904  26.144  34.870  1.00 13.61
ATOM    111  C   GLU  17      14.086  27.474  35.571  1.00  9.38
ATOM    112  O   GLU  17      14.331  27.524  36.765  1.00 15.74
ATOM    113  CB  GLU  17      12.650  25.402  35.344  1.00 14.15
ATOM    114  CG  GLU  17      12.436  24.178  34.447  1.00 15.37
ATOM    115  CD  GLU  17      11.865  24.573  33.105  1.00 49.50
ATOM    116  OE1 GLU  17      11.160  25.557  32.950  1.00 83.46
ATOM    117  OE2 GLU  17      12.220  23.766  32.127  1.00 38.75
ATOM    118  N   LEU  18      13.990  28.571  34.805  1.00 17.82
ATOM    119  CA  LEU  18      14.116  29.914  35.401  1.00 16.61
ATOM    120  C   LEU  18      12.962  30.855  35.057  1.00 14.91
ATOM    121  O   LEU  18      12.585  30.978  33.917  1.00 14.31
ATOM    122  CB  LEU  18      15.426  30.630  35.005  1.00 13.56
ATOM    123  CG  LEU  18      15.533  32.049  35.579  1.00 19.27
ATOM    124  CD1 LEU  18      16.740  32.182  36.489  1.00 21.40
ATOM    125  CD2 LEU  18      15.682  33.033  34.438  1.00 18.38
ATOM    126  N   ASP  19      12.480  31.551  36.082  1.00 17.38
ATOM    127  CA  ASP  19      11.476  32.577  35.940  1.00 19.57
```

FIG 5-3

ATOM	128	C	ASP	19	12.098	33.896	36.360	1.00	11.65
ATOM	129	O	ASP	19	12.486	34.044	37.493	1.00	16.82
ATOM	130	CB	ASP	19	10.234	32.305	36.847	1.00	24.92
ATOM	131	CG	ASP	19	9.305	31.262	36.282	1.00	38.46
ATOM	132	OD1	ASP	19	8.572	30.587	36.989	1.00	61.49
ATOM	133	OD2	ASP	19	9.337	31.189	34.949	1.00	22.44
ATOM	134	N	GLY	20	12.178	34.863	35.471	1.00	16.82
ATOM	135	CA	GLY	20	12.784	36.101	35.908	1.00	19.52
ATOM	136	C	GLY	20	12.048	37.385	35.538	1.00	19.35
ATOM	137	O	GLY	20	11.240	37.443	34.628	1.00	18.22
ATOM	138	N	ASP	21	12.401	38.407	36.286	1.00	13.19
ATOM	139	CA	ASP	21	11.908	39.737	36.112	1.00	16.36
ATOM	140	C	ASP	21	13.039	40.683	36.424	1.00	12.77
ATOM	141	O	ASP	21	13.517	40.742	37.569	1.00	15.18
ATOM	142	CB	ASP	21	10.701	40.036	37.040	1.00	22.26
ATOM	143	CG	ASP	21	10.230	41.491	37.022	1.00	30.80
ATOM	144	OD1	ASP	21	10.878	42.407	36.557	1.00	27.40
ATOM	145	OD2	ASP	21	9.062	41.658	37.604	1.00	45.92
ATOM	146	N	VAL	22	13.464	41.393	35.397	1.00	19.66
ATOM	147	CA	VAL	22	14.524	42.388	35.542	1.00	25.10
ATOM	148	C	VAL	22	14.010	43.780	35.154	1.00	18.26
ATOM	149	O	VAL	22	13.769	44.062	33.955	1.00	15.10
ATOM	150	CB	VAL	22	15.803	42.012	34.750	1.00	26.57
ATOM	151	CG1	VAL	22	16.861	43.127	34.896	1.00	24.27
ATOM	152	CG2	VAL	22	16.365	40.710	35.297	1.00	22.98
ATOM	153	N	ASN	23	13.823	44.641	36.166	1.00	29.32
ATOM	154	CA	ASN	23	13.319	45.993	35.908	1.00	32.81
ATOM	155	C	ASN	23	11.987	45.958	35.142	1.00	32.77
ATOM	156	O	ASN	23	11.774	46.730	34.187	1.00	30.47
ATOM	157	CB	ASN	23	14.344	46.831	35.096	1.00	31.26
ATOM	158	CG	ASN	23	15.374	47.607	35.938	1.00	24.72
ATOM	159	OD1	ASN	23	15.795	47.183	37.024	1.00	27.22
ATOM	160	ND2	ASN	23	15.829	48.723	35.389	1.00	41.15
ATOM	161	N	GLY	24	11.118	45.024	35.519	1.00	24.95
ATOM	162	CA	GLY	24	9.831	44.919	34.848	1.00	23.22
ATOM	163	C	GLY	24	9.832	44.111	33.573	1.00	23.31
ATOM	164	O	GLY	24	8.780	43.868	33.024	1.00	28.37
ATOM	165	N	HIS	25	11.000	43.691	33.071	1.00	20.89
ATOM	166	CA	HIS	25	11.042	42.840	31.877	1.00	19.30
ATOM	167	C	HIS	25	10.981	41.373	32.316	1.00	27.26
ATOM	168	O	HIS	25	11.898	40.850	32.951	1.00	25.47
ATOM	169	CB	HIS	25	12.268	43.060	30.958	1.00	24.20
ATOM	170	CG	HIS	25	12.313	44.382	30.218	1.00	33.04
ATOM	171	ND1	HIS	25	12.917	45.514	30.758	1.00	37.58
ATOM	172	CD2	HIS	25	11.876	44.716	28.971	1.00	42.76
ATOM	173	CE1	HIS	25	12.801	46.497	29.867	1.00	39.14
ATOM	174	NE2	HIS	25	12.185	46.050	28.778	1.00	42.80
ATOM	175	N	LYS	26	9.872	40.728	32.028	1.00	25.90
ATOM	176	CA	LYS	26	9.675	39.355	32.446	1.00	26.27
ATOM	177	C	LYS	26	10.154	38.361	31.429	1.00	27.09
ATOM	178	O	LYS	26	10.027	38.576	30.232	1.00	25.75
ATOM	179	CB	LYS	26	8.230	39.069	32.863	1.00	27.58
ATOM	180	CG	LYS	26	7.873	39.770	34.166	1.00	44.94
ATOM	181	CD	LYS	26	6.369	39.914	34.400	1.00	71.44
ATOM	182	CE	LYS	26	6.008	41.000	35.421	1.00	45.29
ATOM	183	N	PHE	27	10.703	37.250	31.910	1.00	22.04
ATOM	184	CA	PHE	27	11.164	36.236	30.978	1.00	18.78
ATOM	185	C	PHE	27	11.273	34.863	31.619	1.00	14.75
ATOM	186	O	PHE	27	11.293	34.722	32.842	1.00	15.94
ATOM	187	CB	PHE	27	12.495	36.638	30.287	1.00	21.58
ATOM	188	CG	PHE	27	13.599	36.826	31.311	1.00	22.06
ATOM	189	CD1	PHE	27	14.490	35.791	31.612	1.00	23.61
ATOM	190	CD2	PHE	27	13.722	38.029	32.005	1.00	17.85
ATOM	191	CE1	PHE	27	15.487	35.963	32.579	1.00	16.61
ATOM	192	CE2	PHE	27	14.747	38.234	32.931	1.00	19.75
ATOM	193	CZ	PHE	27	15.621	37.187	33.234	1.00	13.83
ATOM	194	N	SER	28	11.370	33.857	30.752	1.00	12.40

FIG 5-4

ATOM	195	CA	SER	28	11.492	32.479	31.186	1.00	15.59
ATOM	196	C	SER	28	12.579	31.749	30.379	1.00	15.96
ATOM	197	O	SER	28	12.699	31.933	29.167	1.00	18.99
ATOM	198	CB	SER	28	10.143	31.702	31.086	1.00	14.48
ATOM	199	OG	SER	28	9.510	31.678	32.353	1.00	31.95
ATOM	200	N	VAL	29	13.335	30.902	31.073	1.00	16.73
ATOM	201	CA	VAL	29	14.361	30.093	30.435	1.00	14.06
ATOM	202	C	VAL	29	14.258	28.614	30.817	1.00	6.80
ATOM	203	O	VAL	29	14.058	28.266	31.987	1.00	10.85
ATOM	204	CB	VAL	29	15.768	30.570	30.839	1.00	17.96
ATOM	205	CG1	VAL	29	16.826	29.599	30.234	1.00	15.30
ATOM	206	CG2	VAL	29	15.989	32.001	30.357	1.00	16.37
ATOM	207	N	SER	30	14.462	27.781	29.824	1.00	11.31
ATOM	208	CA	SER	30	14.535	26.351	30.011	1.00	17.96
ATOM	209	C	SER	30	15.917	25.818	29.571	1.00	11.26
ATOM	210	O	SER	30	16.398	26.157	28.513	1.00	13.17
ATOM	211	CB	SER	30	13.471	25.603	29.202	1.00	19.91
ATOM	212	OG	SER	30	12.249	25.667	29.882	1.00	48.74
ATOM	213	N	GLY	31	16.480	24.926	30.364	1.00	9.88
ATOM	214	CA	GLY	31	17.718	24.321	29.977	1.00	12.44
ATOM	215	C	GLY	31	17.737	22.816	30.249	1.00	13.16
ATOM	216	O	GLY	31	17.149	22.324	31.176	1.00	12.41
ATOM	217	N	GLU	32	18.459	22.112	29.433	1.00	13.44
ATOM	218	CA	GLU	32	18.622	20.670	29.570	1.00	13.73
ATOM	219	C	GLU	32	20.079	20.297	29.262	1.00	17.33
ATOM	220	O	GLU	32	20.734	20.946	28.456	1.00	15.56
ATOM	221	CB	GLU	32	17.761	19.893	28.543	1.00	12.67
ATOM	222	CG	GLU	32	16.264	20.187	28.618	1.00	26.43
ATOM	223	CD	GLU	32	15.501	19.547	27.468	1.00	21.13
ATOM	224	OE1	GLU	32	15.996	18.767	26.698	1.00	23.45
ATOM	225	OE2	GLU	32	14.292	20.022	27.337	1.00	30.63
ATOM	226	N	GLY	33	20.534	19.207	29.822	1.00	15.36
ATOM	227	CA	GLY	33	21.860	18.687	29.518	1.00	12.84
ATOM	228	C	GLY	33	22.236	17.602	30.467	1.00	14.69
ATOM	229	O	GLY	33	21.390	16.919	31.011	1.00	13.56
ATOM	230	N	GLU	34	23.525	17.453	30.702	1.00	15.15
ATOM	231	CA	GLU	34	23.971	16.450	31.621	1.00	18.14
ATOM	232	C	GLU	34	25.220	16.874	32.367	1.00	16.26
ATOM	233	O	GLU	34	25.926	17.760	31.944	1.00	18.67
ATOM	234	CB	GLU	34	24.180	15.114	30.927	1.00	22.53
ATOM	235	CG	GLU	34	24.948	15.261	29.624	1.00	33.78
ATOM	236	CD	GLU	34	24.879	14.020	28.796	1.00	55.15
ATOM	237	OE1	GLU	34	25.861	13.352	28.534	1.00	45.39
ATOM	238	OE2	GLU	34	23.653	13.719	28.430	1.00	56.26
ATOM	239	N	GLY	35	25.461	16.222	33.485	1.00	11.20
ATOM	240	CA	GLY	35	26.611	16.502	34.315	1.00	10.62
ATOM	241	C	GLY	35	27.293	15.192	34.662	1.00	19.92
ATOM	242	O	GLY	35	26.650	14.161	34.750	1.00	16.69
ATOM	243	N	ASP	36	28.594	15.238	34.860	1.00	16.92
ATOM	244	CA	ASP	36	29.367	14.061	35.221	1.00	16.19
ATOM	245	C	ASP	36	30.396	14.505	36.233	1.00	13.94
ATOM	246	O	ASP	36	31.469	15.004	35.879	1.00	15.77
ATOM	247	CB	ASP	36	30.032	13.457	33.948	1.00	19.98
ATOM	248	CG	ASP	36	30.681	12.066	34.075	1.00	31.92
ATOM	249	OD1	ASP	36	31.236	11.519	33.141	1.00	30.97
ATOM	250	OD2	ASP	36	30.587	11.515	35.248	1.00	25.32
ATOM	251	N	ALA	37	30.015	14.402	37.490	1.00	13.40
ATOM	252	CA	ALA	37	30.818	14.846	38.582	1.00	12.98
ATOM	253	C	ALA	37	32.181	14.145	38.637	1.00	21.94
ATOM	254	O	ALA	37	33.084	14.604	39.331	1.00	13.61
ATOM	255	CB	ALA	37	30.070	14.741	39.916	1.00	11.49
ATOM	256	N	THR	38	32.307	13.016	37.945	1.00	15.63
ATOM	257	CA	THR	38	33.581	12.280	37.943	1.00	19.94
ATOM	258	C	THR	38	34.705	13.114	37.335	1.00	25.61
ATOM	259	O	THR	38	35.850	13.069	37.775	1.00	17.99
ATOM	260	CB	THR	38	33.462	12.398	37.299	1.00	22.57
ATOM	261	OG1	THR	38	32.543	13.146	38.067	1.00	29.86

FIG 5-5

```
ATOM    262  CG2 THR    38      34.821  10.213  37.355  1.00 22.90
ATOM    263  N   TYR    39      34.323  13.920  36.347  1.00 18.45
ATOM    264  CA  TYR    39      35.210  14.837  35.675  1.00  9.39
ATOM    265  C   TYR    39      34.874  16.291  35.991  1.00 14.41
ATOM    266  O   TYR    39      35.454  17.177  35.410  1.00 16.24
ATOM    267  CB  TYR    39      35.156  14.582  34.180  1.00 11.82
ATOM    268  CG  TYR    39      35.426  13.137  33.929  1.00 28.73
ATOM    269  CD1 TYR    39      36.715  12.633  34.065  1.00 33.75
ATOM    270  CD2 TYR    39      34.392  12.249  33.642  1.00 39.19
ATOM    271  CE1 TYR    39      36.982  11.276  33.828  1.00 29.75
ATOM    272  CE2 TYR    39      34.635  10.885  33.435  1.00 45.41
ATOM    273  CZ  TYR    39      35.943  10.410  33.570  1.00 57.62
ATOM    274  OH  TYR    39      36.199   9.070  33.364  1.00 70.77
ATOM    275  N   GLY    40      33.935  16.525  36.929  1.00  9.94
ATOM    276  CA  GLY    40      33.474  17.879  37.266  1.00  7.02
ATOM    277  C   GLY    40      32.952  18.600  36.004  1.00  9.45
ATOM    278  O   GLY    40      33.068  19.830  35.829  1.00 12.63
ATOM    279  N   LYS    41      32.380  17.823  35.092  1.00  5.44
ATOM    280  CA  LYS    41      31.954  18.335  33.842  1.00  6.63
ATOM    281  C   LYS    41      30.414  18.554  33.703  1.00 20.92
ATOM    282  O   LYS    41      29.617  17.693  34.085  1.00 12.94
ATOM    283  CB  LYS    41      32.360  17.357  32.827  1.00  8.27
ATOM    284  CG  LYS    41      32.099  17.771  31.419  1.00 13.19
ATOM    285  CD  LYS    41      32.521  16.644  30.481  1.00 20.20
ATOM    286  CE  LYS    41      32.690  17.068  29.032  1.00 35.79
ATOM    287  NZ  LYS    41      33.113  15.954  28.147  1.00 47.56
ATOM    288  N   LEU    42      30.049  19.684  33.069  1.00 18.31
ATOM    289  CA  LEU    42      28.643  20.064  32.794  1.00 16.08
ATOM    290  C   LEU    42      28.456  20.422  31.330  1.00 14.23
ATOM    291  O   LEU    42      29.240  21.168  30.787  1.00 14.79
ATOM    292  CB  LEU    42      28.223  21.300  33.621  1.00 13.22
ATOM    293  CG  LEU    42      28.007  21.061  35.082  1.00 16.70
ATOM    294  CD1 LEU    42      27.894  22.406  35.782  1.00 13.79
ATOM    295  CD2 LEU    42      26.732  20.243  35.295  1.00 18.70
ATOM    296  N   THR    43      27.395  19.914  30.672  1.00  8.04
ATOM    297  CA  THR    43      27.103  20.275  29.282  1.00  4.87
ATOM    298  C   THR    43      25.636  20.666  29.186  1.00 17.23
ATOM    299  O   THR    43      24.811  19.818  29.442  1.00 14.38
ATOM    300  CB  THR    43      27.351  19.140  28.317  1.00 21.59
ATOM    301  OG1 THR    43      28.692  18.743  28.415  1.00 42.74
ATOM    302  CG2 THR    43      27.073  19.675  26.917  1.00 31.23
ATOM    303  N   LEU    44      25.327  21.934  29.830  1.00 11.83
ATOM    304  CA  LEU    44      23.944  22.409  29.847  1.00 13.81
ATOM    305  C   LEU    44      23.589  23.307  28.668  1.00 18.19
ATOM    306  O   LEU    44      24.416  23.989  28.107  1.00 13.86
ATOM    307  CB  LEU    44      23.725  23.275  30.125  1.00 15.37
ATOM    308  CG  LEU    44      23.369  22.584  31.456  1.00 24.69
ATOM    309  CD1 LEU    44      21.869  22.381  31.601  1.00 23.20
ATOM    310  CD2 LEU    44      24.083  21.286  31.650  1.00 46.18
ATOM    311  N   LYS    45      22.294  23.331  27.339  1.00 10.29
ATOM    312  CA  LYS    45      21.752  24.224  26.358  1.00 11.94
ATOM    313  C   LYS    45      20.534  24.913  26.957  1.00 19.35
ATOM    314  O   LYS    45      19.665  24.248  27.530  1.00 18.43
ATOM    315  CB  LYS    45      21.409  23.560  25.060  1.00 13.75
ATOM    316  CG  LYS    45      20.878  24.556  24.045  1.00  8.83
ATOM    317  CD  LYS    45      20.486  23.863  22.746  1.00 26.87
ATOM    318  CE  LYS    45      19.574  24.688  21.842  1.00 16.58
ATOM    319  NZ  LYS    45      19.318  24.024  20.555  1.00 18.33
ATOM    320  N   PHE    46      20.535  26.236  26.910  1.00 12.34
ATOM    321  CA  PHE    46      19.463  27.048  27.451  1.00 13.32
ATOM    322  C   PHE    46      18.759  27.719  26.343  1.00 18.26
ATOM    323  O   PHE    46      19.386  28.093  25.360  1.00 16.83
ATOM    324  CB  PHE    46      19.934  28.101  28.473  1.00 15.29
ATOM    325  CG  PHE    46      20.773  27.495  29.552  1.00 13.81
ATOM    326  CD1 PHE    46      22.132  27.268  29.337  1.00 17.06
ATOM    327  CD2 PHE    46      20.209  27.121  30.774  1.00  8.24
ATOM    328  CE1 PHE    46      22.924  26.693  30.331  1.00 15.95
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FIG 5-6

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ATOM    329  CE2 PHE     46      20.979  26.524  31.767  1.00 11.90
ATOM    330  CZ  PHE     46      22.340  26.309  31.540  1.00  8.84
ATOM    331  N   ILE     47      17.440  27.845  26.498  1.00 13.24
ATOM    332  CA  ILE     47      16.588  28.453  25.479  1.00 18.02
ATOM    333  C   ILE     47      15.645  29.460  26.118  1.00 20.14
ATOM    334  O   ILE     47      15.039  29.162  27.148  1.00 17.67
ATOM    335  CB  ILE     47      15.737  27.386  24.801  1.00 22.67
ATOM    336  CG1 ILE     47      16.585  26.271  24.291  1.00 20.66
ATOM    337  CG2 ILE     47      15.024  28.002  23.641  1.00 33.79
ATOM    338  CD1 ILE     47      16.639  26.293  22.805  1.00 23.69
ATOM    339  N   CYS     48      15.564  30.653  25.561  1.00 14.68
ATOM    340  CA  CYS     48      14.681  31.635  26.170  1.00 16.93
ATOM    341  C   CYS     48      13.323  31.352  25.628  1.00 24.18
ATOM    342  O   CYS     48      13.122  31.513  24.453  1.00 20.63
ATOM    343  CB  CYS     48      15.063  33.116  25.885  1.00 16.85
ATOM    344  SG  CYS     48      13.913  34.268  26.712  1.00 22.06
ATOM    345  N   THR     49      12.424  30.871  26.484  1.00 27.31
ATOM    346  CA  THR     49      11.101  30.458  26.042  1.00 32.18
ATOM    347  C   THR     49      10.106  31.572  25.803  1.00 37.51
ATOM    348  O   THR     49       9.150  31.407  25.061  1.00 35.71
ATOM    349  CB  THR     49      10.537  29.417  26.972  1.00 23.66
ATOM    350  OG1 THR     49      10.387  29.989  28.258  1.00 30.10
ATOM    351  CG2 THR     49      11.512  28.226  27.022  1.00 29.98
ATOM    352  N   THR     50      10.314  32.693  26.447  1.00 32.34
ATOM    353  CA  THR     50       9.416  33.810  26.283  1.00 28.67
ATOM    354  C   THR     50       9.836  34.711  25.126  1.00 37.98
ATOM    355  O   THR     50       9.228  35.763  24.904  1.00 39.17
ATOM    356  CB  THR     50       9.251  34.611  27.589  1.00 36.23
ATOM    357  OG1 THR     50      10.512  34.980  28.118  1.00 35.37
ATOM    358  CG2 THR     50       8.507  33.773  28.602  1.00 27.78
ATOM    359  N   GLY     51      10.881  34.282  24.372  1.00 31.04
ATOM    360  CA  GLY     51      11.394  35.059  23.239  1.00 32.42
ATOM    361  C   GLY     51      12.865  35.542  23.427  1.00 48.45
ATOM    362  O   GLY     51      13.779  34.737  23.701  1.00 57.11
ATOM    363  N   LYS     52      13.087  36.862  23.282  1.00 36.08
ATOM    364  CA  LYS     52      14.416  37.460  23.415  1.00 35.75
ATOM    365  C   LYS     52      14.827  37.726  24.861  1.00 29.65
ATOM    366  O   LYS     52      14.140  38.420  25.620  1.00 25.70
ATOM    367  CB  LYS     52      14.577  38.714  22.582  1.00 43.37
ATOM    368  CG  LYS     52      15.772  38.649  21.644  1.00 78.17
ATOM    369  N   LEU     53      15.983  37.190  25.250  1.00 19.22
ATOM    370  CA  LEU     53      16.439  37.430  26.596  1.00 13.52
ATOM    371  C   LEU     53      16.717  38.932  26.775  1.00 17.76
ATOM    372  O   LEU     53      17.392  39.539  25.973  1.00 21.59
ATOM    373  CB  LEU     53      17.705  36.567  26.845  1.00 17.39
ATOM    374  CG  LEU     53      18.100  36.435  28.302  1.00 17.43
ATOM    375  CD1 LEU     53      17.048  35.621  29.053  1.00 20.12
ATOM    376  CD2 LEU     53      19.440  35.718  28.368  1.00 16.11
ATOM    377  N   PRO     54      16.197  39.525  27.817  1.00 16.69
ATOM    378  CA  PRO     54      16.324  40.962  28.092  1.00 18.60
ATOM    379  C   PRO     54      17.638  41.414  28.707  1.00 25.39
ATOM    380  O   PRO     54      17.865  42.609  28.861  1.00 18.88
ATOM    381  CB  PRO     54      15.268  41.265  29.139  1.00 22.52
ATOM    382  CG  PRO     54      14.832  39.933  29.720  1.00 26.02
ATOM    383  CD  PRO     54      15.318  38.855  28.779  1.00 21.26
ATOM    384  N   VAL     55      18.435  40.455  29.151  1.00 23.32
ATOM    385  CA  VAL     55      19.746  40.716  29.711  1.00 15.83
ATOM    386  C   VAL     55      20.688  39.868  28.973  1.00 19.38
ATOM    387  O   VAL     55      20.268  39.035  28.219  1.00 20.34
ATOM    388  CB  VAL     55      19.814  40.409  31.147  1.00 17.67
ATOM    389  CG1 VAL     55      18.864  41.340  31.851  1.00 22.52
ATOM    390  CG2 VAL     55      19.402  38.959  31.397  1.00 19.11
ATOM    391  N   PRO     56      21.963  40.070  29.167  1.00 19.37
ATOM    392  CA  PRO     56      22.911  39.259  28.447  1.00 13.09
ATOM    393  C   PRO     56      23.059  37.834  29.038  1.00  9.83
ATOM    394  O   PRO     56      23.067  37.631  30.254  1.00 12.35
ATOM    395  CB  PRO     56      24.231  40.062  28.420  1.00 18.34
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FIG 5-7

ATOM	396	CG	PRO	56	23.851	41.478	28.849	1.00	20.73
ATOM	397	CD	PRO	56	22.525	41.379	29.578	1.00	18.66
ATOM	398	N	TRP	57	23.202	36.848	28.158	1.00	11.12
ATOM	399	CA	TRP	57	23.364	35.458	28.595	1.00	12.55
ATOM	400	C	TRP	57	24.411	35.239	29.700	1.00	14.13
ATOM	401	O	TRP	57	24.178	34.586	30.709	1.00	11.49
ATOM	402	CB	TRP	57	23.604	34.535	27.406	1.00	10.56
ATOM	403	CG	TRP	57	22.335	34.237	26.641	1.00	12.65
ATOM	404	CD1	TRP	57	21.999	34.714	25.426	1.00	16.24
ATOM	405	CD2	TRP	57	21.281	33.327	27.013	1.00	12.50
ATOM	406	NE1	TRP	57	20.784	34.200	25.018	1.00	14.25
ATOM	407	CE2	TRP	57	20.315	33.354	25.963	1.00	14.65
ATOM	408	CE3	TRP	57	21.052	32.521	28.129	1.00	12.01
ATOM	409	CZ2	TRP	57	19.148	32.583	26.007	1.00	14.36
ATOM	410	CZ3	TRP	57	19.887	31.767	28.170	1.00	14.23
ATOM	411	CH2	TRP	57	18.945	31.818	27.128	1.00	10.01
ATOM	412	N	PRO	58	25.594	35.800	29.518	1.00	15.78
ATOM	413	CA	PRO	58	26.629	35.616	30.503	1.00	9.53
ATOM	414	C	PRO	58	26.241	36.010	31.878	1.00	9.71
ATOM	415	O	PRO	58	26.760	35.467	32.825	1.00	11.70
ATOM	416	CB	PRO	58	27.833	36.441	30.040	1.00	10.83
ATOM	417	CG	PRO	58	27.597	36.748	28.582	1.00	18.50
ATOM	418	CD	PRO	58	26.137	36.432	28.278	1.00	15.82
ATOM	419	N	THR	59	25.336	36.977	32.021	1.00	7.54
ATOM	420	CA	THR	59	24.976	37.366	33.357	1.00	4.53
ATOM	421	C	THR	59	24.228	36.258	34.137	1.00	8.41
ATOM	422	O	THR	59	24.174	36.261	35.367	1.00	10.57
ATOM	423	CB	THR	59	24.197	38.691	33.384	1.00	16.64
ATOM	424	OG1	THR	59	22.895	38.480	32.844	1.00	15.51
ATOM	425	CG2	THR	59	24.917	39.731	32.542	1.00	15.76
ATOM	426	N	LEU	60	23.686	35.304	33.427	1.00	11.99
ATOM	427	CA	LEU	60	22.859	34.248	34.073	1.00	9.15
ATOM	428	C	LEU	60	23.657	32.944	34.385	1.00	15.62
ATOM	429	O	LEU	60	23.118	32.027	35.042	1.00	11.99
ATOM	430	CB	LEU	60	21.645	33.914	33.203	1.00	7.67
ATOM	431	CG	LEU	60	20.728	35.111	33.042	1.00	14.06
ATOM	432	CD1	LEU	60	19.620	34.775	32.062	1.00	14.54
ATOM	433	CD2	LEU	60	20.142	35.456	34.394	1.00	10.67
ATOM	434	N	VAL	61	24.893	32.837	33.917	1.00	11.27
ATOM	435	CA	VAL	61	25.636	31.587	34.094	1.00	4.37
ATOM	436	C	VAL	61	25.678	31.013	35.496	1.00	6.02
ATOM	437	O	VAL	61	25.355	29.805	35.743	1.00	10.75
ATOM	438	CB	VAL	61	27.050	31.643	33.406	1.00	7.14
ATOM	439	CG1	VAL	61	27.888	30.396	33.805	1.00	6.47
ATOM	440	CG2	VAL	61	26.890	31.745	31.876	1.00	6.63
ATOM	441	N	THR	62	26.053	31.843	36.442	1.00	7.02
ATOM	442	CA	THR	62	26.178	31.421	37.808	1.00	6.51
ATOM	443	C	THR	62	24.862	30.954	38.410	1.00	9.22
ATOM	444	O	THR	62	24.801	30.163	39.352	1.00	6.99
ATOM	445	CB	THR	62	26.816	32.520	38.660	1.00	16.97
ATOM	446	OG1	THR	62	26.103	33.744	38.453	1.00	12.00
ATOM	447	CG2	THR	62	28.297	32.708	38.225	1.00	8.86
ATOM	448	N	THR	63	23.814	31.547	37.910	1.00	9.98
ATOM	449	CA	THR	63	22.457	31.212	38.388	1.00	6.59
ATOM	450	C	THR	63	22.033	29.830	37.865	1.00	8.14
ATOM	451	O	THR	63	21.499	28.984	38.604	1.00	13.48
ATOM	452	CB	THR	63	21.458	32.312	37.925	1.00	11.24
ATOM	453	OG1	THR	63	21.785	33.498	38.602	1.00	11.75
ATOM	454	CG2	THR	63	20.024	31.897	38.296	1.00	9.31
ATOM	455	N	PHE	64	22.250	29.620	36.583	1.00	10.19
ATOM	456	CA	PHE	64	21.895	28.371	35.995	1.00	8.00
ATOM	457	C	PHE	64	22.774	27.253	36.518	1.00	25.26
ATOM	458	O	PHE	64	22.313	26.147	36.761	1.00	9.64
ATOM	459	CB	PHE	64	22.114	28.438	34.513	1.00	8.88
ATOM	460	CG	PHE	64	21.233	29.357	33.750	1.00	10.96
ATOM	461	CD1	PHE	64	21.724	29.954	32.593	1.00	9.15
ATOM	462	CD2	PHE	64	19.899	29.563	34.106	1.00	14.43

FIG 5-8

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ATOM    463  CE1 PHE    64      20.936  30.792  31.805  1.00 14.20
ATOM    464  CE2 PHE    64      19.077  30.375  33.317  1.00 13.95
ATOM    465  CZ  PHE    64      19.597  30.983  32.171  1.00 16.35
HETATM  466  N1  CRO    66      24.077  27.513  36.610  1.00 11.86
HETATM  467  CG1 CRO    66      25.155  25.422  34.796  1.00 16.67
HETATM  468  OG1 CRO    66      26.679  27.129  35.461  1.00 14.22
HETATM  469  CB1 CRO    66      25.931  26.035  35.930  1.00 10.77
HETATM  470  CA1 CRO    66      25.011  26.478  37.078  1.00  7.34
HETATM  471  C1  CRO    66      25.718  26.991  38.253  1.00 17.70
HETATM  472  N2  CRO    66      26.975  27.732  38.216  1.00  9.21
HETATM  473  OH  CRO    66      32.894  30.804  36.971  1.00 13.84
HETATM  474  CD2 CRO    66      30.487  30.110  39.805  1.00 10.79
HETATM  475  CE2 CRO    66      31.614  30.563  39.085  1.00 10.01
HETATM  476  CZ  CRO    66      31.718  30.300  37.721  1.00  9.48
HETATM  477  CE1 CRO    66      30.707  29.546  37.033  1.00 17.44
HETATM  478  CD1 CRO    66      29.541  29.103  37.742  1.00 11.31
HETATM  479  CG2 CRO    66      29.437  29.370  39.124  1.00  7.67
HETATM  480  CB2 CRO    66      28.329  28.822  39.960  1.00 10.75
HETATM  481  CA2 CRO    66      27.197  28.245  39.512  1.00 16.08
HETATM  482  C2  CRO    66      26.043  27.875  40.370  1.00  5.46
HETATM  483  O2  CRO    66      26.022  27.962  41.566  1.00 13.20
HETATM  484  N3  CRO    66      25.240  26.978  39.517  1.00 18.43
HETATM  485  CA3 CRO    66      23.840  26.511  39.734  1.00 10.40
HETATM  486  C3  CRO    66      23.413  25.550  40.817  1.00 11.96
HETATM  487  O3  CRO    66      22.747  26.014  41.764  1.00100.00
ATOM    488  N   VAL    68      23.737  24.208  41.005  1.00 29.95
ATOM    489  CA  VAL    68      24.209  22.972  40.304  1.00 17.16
ATOM    490  C   VAL    68      25.692  22.550  40.734  1.00 14.88
ATOM    491  O   VAL    68      26.378  21.821  40.026  1.00  9.03
ATOM    492  CB  VAL    68      23.870  22.899  38.831  1.00 18.94
ATOM    493  CG1 VAL    68      24.685  22.088  37.942  1.00 17.17
ATOM    494  CG2 VAL    68      22.396  22.538  38.680  1.00 18.80
ATOM    495  N   GLN    69      26.129  22.965  41.914  1.00 11.04
ATOM    496  CA  GLN    69      27.465  22.764  42.394  1.00 15.00
ATOM    497  C   GLN    69      27.749  21.366  42.893  1.00 22.46
ATOM    498  O   GLN    69      28.876  21.025  43.154  1.00 15.84
ATOM    499  CB  GLN    69      27.929  23.852  43.414  1.00 10.93
ATOM    500  CG  GLN    69      28.202  25.174  42.615  1.00 14.13
ATOM    501  CD  GLN    69      28.216  26.385  43.520  1.00 17.01
ATOM    502  OE1 GLN    69      27.433  26.476  44.448  1.00 18.94
ATOM    503  NE2 GLN    69      29.151  27.300  43.241  1.00  8.52
ATOM    504  N   CYS    70      26.703  20.540  42.906  1.00 12.10
ATOM    505  CA  CYS    70      26.862  19.171  43.287  1.00 11.84
ATOM    506  C   CYS    70      27.611  18.391  42.175  1.00 10.54
ATOM    507  O   CYS    70      28.036  17.242  42.367  1.00 14.70
ATOM    508  CB  CYS    70      25.476  18.584  43.596  1.00 14.52
ATOM    509  SG  CYS    70      24.325  19.012  42.251  1.00 15.61
ATOM    510  N   PHE    71      27.801  19.029  41.005  1.00  8.64
ATOM    511  CA  PHE    71      28.525  18.419  39.883  1.00  6.59
ATOM    512  C   PHE    71      30.041  18.754  39.876  1.00 16.43
ATOM    513  O   PHE    71      30.753  18.481  38.916  1.00 13.05
ATOM    514  CB  PHE    71      27.951  18.771  38.523  1.00  7.61
ATOM    515  CG  PHE    71      26.669  18.016  38.303  1.00 14.73
ATOM    516  CD1 PHE    71      26.693  16.642  38.050  1.00 10.34
ATOM    517  CD2 PHE    71      25.434  18.660  38.453  1.00 17.14
ATOM    518  CE1 PHE    71      25.506  15.931  37.866  1.00 15.09
ATOM    519  CE2 PHE    71      24.238  17.961  38.300  1.00 20.92
ATOM    520  CZ  PHE    71      24.282  16.598  37.990  1.00 18.49
ATOM    521  N   SER    72      30.500  19.370  40.938  1.00 13.13
ATOM    522  CA  SER    72      31.889  19.715  41.075  1.00 11.65
ATOM    523  C   SER    72      32.689  18.446  41.357  1.00 14.56
ATOM    524  O   SER    72      32.256  17.566  42.122  1.00 10.90
ATOM    525  CB  SER    72      32.075  20.672  42.257  1.00  8.65
ATOM    526  OG  SER    72      31.361  21.874  42.038  1.00 19.29
ATOM    527  N   ARG    73      33.905  18.358  40.794  1.00 16.27
ATOM    528  CA  ARG    73      34.695  17.212  41.117  1.00 13.56
ATOM    529  C   ARG    73      35.414  17.426  42.443  1.00 19.96
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FIG 5-9

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ATOM    530  O   ARG  73      36.182  18.376  42.599  1.00 16.14
ATOM    531  CB  ARG  73      35.694  16.817  40.013  1.00 16.80
ATOM    532  CG  ARG  73      36.549  15.616  40.460  1.00 20.13
ATOM    533  CD  ARG  73      37.489  15.093  39.381  1.00 28.47
ATOM    534  NE  ARG  73      38.743  15.859  39.260  1.00 25.48
ATOM    535  CZ  ARG  73      39.756  15.777  40.127  1.00 28.04
ATOM    536  NH1 ARG  73      39.688  15.004  41.195  1.00 28.76
ATOM    537  NH2 ARG  73      40.865  16.504  39.918  1.00 39.65
ATOM    538  N   TYR  74      35.151  16.561  43.424  1.00 12.05
ATOM    539  CA  TYR  74      35.861  16.659  44.690  1.00 11.57
ATOM    540  C   TYR  74      36.946  15.566  44.721  1.00 25.02
ATOM    541  O   TYR  74      36.658  14.387  44.558  1.00 19.71
ATOM    542  CB  TYR  74      34.978  16.528  45.934  1.00 15.51
ATOM    543  CG  TYR  74      34.395  17.850  46.402  1.00 16.59
ATOM    544  CD1 TYR  74      33.455  18.546  45.631  1.00 14.44
ATOM    545  CD2 TYR  74      34.799  18.399  47.618  1.00 15.94
ATOM    546  CE1 TYR  74      32.901  19.756  46.059  1.00  7.99
ATOM    547  CE2 TYR  74      34.261  19.612  48.058  1.00 18.29
ATOM    548  CZ  TYR  74      33.294  20.276  47.298  1.00 13.87
ATOM    549  OH  TYR  74      32.829  21.507  47.738  1.00 18.39
ATOM    550  N   PRO  75      38.181  15.947  44.902  1.00 19.20
ATOM    551  CA  PRO  75      39.213  14.940  44.995  1.00 18.42
ATOM    552  C   PRO  75      38.958  13.993  46.175  1.00 15.60
ATOM    553  O   PRO  75      38.373  14.361  47.174  1.00 11.99
ATOM    554  CB  PRO  75      40.514  15.681  45.196  1.00 18.31
ATOM    555  CG  PRO  75      40.242  17.158  44.863  1.00 24.81
ATOM    556  CD  PRO  75      38.742  17.306  44.694  1.00 15.41
ATOM    557  N   ASP  76      39.433  12.756  46.038  1.00 18.63
ATOM    558  CA  ASP  76      39.269  11.770  47.062  1.00 16.19
ATOM    559  C   ASP  76      39.581  12.280  48.431  1.00 15.92
ATOM    560  O   ASP  76      38.862  12.042  49.389  1.00 17.35
ATOM    561  CB  ASP  76      40.083  10.507  46.790  1.00 18.69
ATOM    562  CG  ASP  76      39.826   9.432  47.825  1.00 24.04
ATOM    563  OD1 ASP  76      40.523   9.268  48.817  1.00 29.72
ATOM    564  OD2 ASP  76      38.732   8.743  47.584  1.00 40.96
ATOM    565  N   HIS  77      40.647  12.984  48.561  1.00 18.79
ATOM    566  CA  HIS  77      40.978  13.418  49.877  1.00 19.36
ATOM    567  C   HIS  77      40.117  14.507  50.397  1.00 24.57
ATOM    568  O   HIS  77      40.205  14.826  51.551  1.00 27.15
ATOM    569  CB  HIS  77      42.435  13.806  50.042  1.00 19.84
ATOM    570  CG  HIS  77      42.743  15.035  49.322  1.00 17.31
ATOM    571  ND1 HIS  77      42.925  15.028  47.953  1.00 21.86
ATOM    572  CD2 HIS  77      42.925  16.295  49.774  1.00 18.70
ATOM    573  CE1 HIS  77      43.203  16.289  47.593  1.00 17.49
ATOM    574  NE2 HIS  77      43.213  17.069  48.668  1.00 18.11
ATOM    575  N   MSE  78      39.277  15.069  49.565  1.00 25.36
ATOM    576  CA  MSE  78      38.412  16.140  50.026  1.00 24.65
ATOM    577  C   MSE  78      36.920  15.774  50.066  1.00 26.47
ATOM    578  O   MSE  78      36.070  16.636  50.260  1.00 28.16
ATOM    579  CB  MSE  78      38.596  17.331  49.121  1.00 26.38
ATOM    580  CG  MSE  78      39.803  18.177  49.406  1.00 27.01
ATOM    581  SE  MSE  78      39.987  19.608  48.117  1.00 43.09
ATOM    582  CE  MSE  78      38.874  20.873  49.044  1.00 27.11
ATOM    583  N   LYS  79      36.606  14.509  49.856  1.00 18.68
ATOM    584  CA  LYS  79      35.216  14.061  49.853  1.00 21.54
ATOM    585  C   LYS  79      34.406  14.449  51.082  1.00 20.21
ATOM    586  O   LYS  79      33.186  14.652  51.025  1.00 21.08
ATOM    587  CB  LYS  79      35.152  12.581  49.612  1.00 23.48
ATOM    588  CG  LYS  79      35.859  12.225  48.317  1.00 41.09
ATOM    589  CD  LYS  79      35.159  11.134  47.535  1.00 34.66
ATOM    590  CE  LYS  79      35.796  10.881  46.181  1.00 53.46
ATOM    591  NZ  LYS  79      35.084  11.549  45.080  1.00 49.53
ATOM    592  N   ARG  80      35.069  14.542  52.213  1.00 19.77
ATOM    593  CA  ARG  80      34.365  14.874  53.434  1.00 20.13
ATOM    594  C   ARG  80      33.898  16.311  53.481  1.00 26.42
ATOM    595  O   ARG  80      33.251  16.717  54.467  1.00 23.51
ATOM    596  CB  ARG  80      35.155  14.549  54.700  1.00 24.58
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FIG 5-10

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ATOM    597  CG  ARG  80      36.204  15.620  55.034  1.00 29.71
ATOM    598  CD  ARG  80      36.964  15.344  56.335  1.00 61.30
ATOM    599  NE  ARG  80      36.551  16.230  57.415  1.00 71.14
ATOM    600  CZ  ARG  80      37.398  16.882  58.192  1.00100.00
ATOM    601  NH1 ARG  80      38.714  16.758  58.040  1.00100.00
ATOM    602  NH2 ARG  80      36.917  17.679  59.155  1.00 99.06
ATOM    603  N   HIS  81      34.275  17.121  52.473  1.00 18.77
ATOM    604  CA  HIS  81      33.903  18.547  52.499  1.00 19.60
ATOM    605  C   HIS  81      32.841  18.883  51.486  1.00 18.62
ATOM    606  O   HIS  81      32.557  20.043  51.295  1.00 17.76
ATOM    607  CB  HIS  81      35.129  19.472  52.283  1.00 20.39
ATOM    608  CG  HIS  81      36.221  19.224  53.305  1.00 28.02
ATOM    609  ND1 HIS  81      36.127  19.701  54.618  1.00 30.59
ATOM    610  CD2 HIS  81      37.392  18.535  53.202  1.00 29.02
ATOM    611  CE1 HIS  81      37.218  19.308  55.265  1.00 26.24
ATOM    612  NE2 HIS  81      37.991  18.603  54.452  1.00 28.18
ATOM    613  N   ASP  82      32.298  17.843  50.841  1.00 12.20
ATOM    614  CA  ASP  82      31.358  18.011  49.769  1.00 13.24
ATOM    615  C   ASP  82      29.922  18.148  50.259  1.00 24.30
ATOM    616  O   ASP  82      29.175  17.195  50.243  1.00 16.55
ATOM    617  CB  ASP  82      31.480  16.917  48.730  1.00 12.23
ATOM    618  CG  ASP  82      30.642  17.209  47.518  1.00  9.92
ATOM    619  OD1 ASP  82      29.870  18.134  47.459  1.00 20.31
ATOM    620  OD2 ASP  82      30.938  16.466  46.507  1.00 11.12
ATOM    621  N   PHE  83      29.566  19.353  50.705  1.00 23.66
ATOM    622  CA  PHE  83      28.220  19.634  51.201  1.00 20.23
ATOM    623  C   PHE  83      27.154  19.333  50.168  1.00 20.93
ATOM    624  O   PHE  83      26.116  18.733  50.503  1.00 15.97
ATOM    625  CB  PHE  83      28.077  21.106  51.666  1.00 19.59
ATOM    626  CG  PHE  83      26.624  21.613  51.805  1.00 16.91
ATOM    627  CD1 PHE  83      25.946  21.498  53.021  1.00 17.76
ATOM    628  CD2 PHE  83      25.968  22.236  50.734  1.00 18.88
ATOM    629  CE1 PHE  83      24.635  21.960  53.156  1.00 24.13
ATOM    630  CE2 PHE  83      24.650  22.690  50.840  1.00 19.24
ATOM    631  CZ  PHE  83      24.001  22.575  52.068  1.00 20.67
ATOM    632  N   PHE  84      27.432  19.784  48.921  1.00 14.06
ATOM    633  CA  PHE  84      26.515  19.693  47.809  1.00 12.96
ATOM    634  C   PHE  84      25.893  18.332  47.602  1.00 24.96
ATOM    635  O   PHE  84      24.674  18.200  47.534  1.00 21.55
ATOM    636  CB  PHE  84      27.085  20.265  46.513  1.00 13.44
ATOM    637  CG  PHE  84      27.630  21.645  46.721  1.00 14.27
ATOM    638  CD1 PHE  84      29.001  21.845  46.890  1.00 15.17
ATOM    639  CD2 PHE  84      26.781  22.753  46.752  1.00 13.48
ATOM    640  CE1 PHE  84      29.520  23.129  47.073  1.00 14.63
ATOM    641  CE2 PHE  84      27.276  24.041  46.969  1.00 16.34
ATOM    642  CZ  PHE  84      28.650  24.221  47.137  1.00 15.77
ATOM    643  N   LYS  85      26.738  17.330  47.482  1.00 14.07
ATOM    644  CA  LYS  85      26.294  15.985  47.283  1.00 13.30
ATOM    645  C   LYS  85      25.657  15.371  48.547  1.00 13.43
ATOM    646  O   LYS  85      24.773  14.509  48.429  1.00 18.46
ATOM    647  CB  LYS  85      27.434  15.089  46.757  1.00 17.38
ATOM    648  CG  LYS  85      27.873  15.372  45.323  1.00 13.93
ATOM    649  CD  LYS  85      28.969  14.381  44.888  1.00 13.23
ATOM    650  CE  LYS  85      29.766  14.819  43.662  1.00 10.36
ATOM    651  NZ  LYS  85      30.319  16.185  43.773  1.00 12.92
ATOM    652  N   SER  86      26.119  15.795  49.752  1.00 11.03
ATOM    653  CA  SER  86      25.610  15.267  50.998  1.00 12.09
ATOM    654  C   SER  86      24.156  15.639  51.240  1.00 21.58
ATOM    655  O   SER  86      23.452  14.979  52.013  1.00 19.89
ATOM    656  CB  SER  86      26.448  15.661  52.208  1.00 16.45
ATOM    657  OG  SER  86      26.308  17.042  52.495  1.00 22.05
ATOM    658  N   ALA  87      23.705  16.698  50.582  1.00 18.09
ATOM    659  CA  ALA  87      22.333  17.138  50.762  1.00 19.52
ATOM    660  C   ALA  87      21.337  16.399  49.870  1.00 18.60
ATOM    661  O   ALA  87      20.162  16.557  50.040  1.00 19.55
ATOM    662  CB  ALA  87      22.204  18.647  50.632  1.00 19.22
ATOM    663  N   MSE  88      21.835  15.536  48.976  1.00 14.05
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FIG 5-11

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ATOM    664  CA  MSE   88      21.007  14.796  48.035  1.00 15.32
ATOM    665  C   MSE   88      20.496  13.448  48.579  1.00 21.48
ATOM    666  O   MSE   88      21.109  12.876  49.457  1.00 23.03
ATOM    667  CB  MSE   88      21.848  14.593  46.791  1.00 15.98
ATOM    668  CG  MSE   88      22.263  15.891  46.131  1.00 10.66
ATOM    669  SE  MSE   88      20.737  16.894  45.394  1.00 31.99
ATOM    670  CE  MSE   88      21.318  18.684  45.748  1.00 28.66
ATOM    671  N   PRO   89      19.363  12.930  48.084  1.00 14.78
ATOM    672  CA  PRO   89      18.552  13.475  47.008  1.00 14.80
ATOM    673  C   PRO   89      17.572  14.611  47.385  1.00 12.10
ATOM    674  O   PRO   89      17.085  15.301  46.493  1.00 18.06
ATOM    675  CB  PRO   89      17.733  12.294  46.494  1.00 17.00
ATOM    676  CG  PRO   89      17.726  11.261  47.607  1.00 15.83
ATOM    677  CD  PRO   89      18.844  11.642  48.560  1.00 17.16
ATOM    678  N   GLU   90      17.278  14.795  48.695  1.00 14.63
ATOM    679  CA  GLU   90      16.348  15.838  49.157  1.00 20.68
ATOM    680  C   GLU   90      16.701  17.229  48.645  1.00 25.59
ATOM    681  O   GLU   90      15.833  18.042  48.368  1.00 21.57
ATOM    682  CB  GLU   90      16.031  15.816  50.682  1.00 22.21
ATOM    683  CG  GLU   90      15.782  14.403  51.228  1.00 37.69
ATOM    684  CD  GLU   90      17.071  13.641  51.447  1.00 83.49
ATOM    685  OE1 GLU   90      18.179  14.151  51.342  1.00 54.80
ATOM    686  OE2 GLU   90      16.875  12.373  51.749  1.00 64.65
ATOM    687  N   GLY   91      17.977  17.509  48.510  1.00 21.39
ATOM    688  CA  GLY   91      18.394  18.769  47.906  1.00 17.77
ATOM    689  C   GLY   91      18.673  19.911  48.839  1.00 12.17
ATOM    690  O   GLY   91      18.769  19.764  50.055  1.00 16.81
ATOM    691  N   TYR   92      18.861  21.086  48.225  1.00 13.02
ATOM    692  CA  TYR   92      19.143  22.266  48.994  1.00 10.33
ATOM    693  C   TYR   92      18.575  23.478  48.347  1.00  9.87
ATOM    694  O   TYR   92      18.270  23.483  47.144  1.00 15.89
ATOM    695  CB  TYR   92      20.678  22.488  49.278  1.00 15.40
ATOM    696  CG  TYR   92      21.546  22.468  48.012  1.00 15.13
ATOM    697  CD1 TYR   92      21.620  23.576  47.166  1.00 14.75
ATOM    698  CD2 TYR   92      22.317  21.350  47.683  1.00 16.09
ATOM    699  CE1 TYR   92      22.404  23.561  46.009  1.00  6.50
ATOM    700  CE2 TYR   92      23.067  21.300  46.504  1.00 15.12
ATOM    701  CZ  TYR   92      23.156  22.424  45.683  1.00 18.13
ATOM    702  OH  TYR   92      23.944  22.393  44.517  1.00 13.37
ATOM    703  N   VAL   93      18.447  24.504  49.189  1.00 11.93
ATOM    704  CA  VAL   93      18.025  25.822  48.778  1.00 14.74
ATOM    705  C   VAL   93      19.281  26.666  48.625  1.00 16.00
ATOM    706  O   VAL   93      20.172  26.625  49.451  1.00 15.16
ATOM    707  CB  VAL   93      17.073  26.480  49.791  1.00 23.45
ATOM    708  CG1 VAL   93      16.855  27.937  49.413  1.00 26.05
ATOM    709  CG2 VAL   93      15.716  25.764  49.771  1.00 22.90
ATOM    710  N   GLN   94      19.361  27.345  47.521  1.00 13.78
ATOM    711  CA  GLN   94      20.480  28.195  47.227  1.00 10.53
ATOM    712  C   GLN   94      19.948  29.583  46.998  1.00 12.23
ATOM    713  O   GLN   94      19.153  29.788  46.061  1.00 15.52
ATOM    714  CB  GLN   94      21.232  27.727  45.934  1.00  7.95
ATOM    715  CG  GLN   94      22.361  28.708  45.469  1.00 11.87
ATOM    716  CD  GLN   94      23.431  27.999  44.632  1.00 12.04
ATOM    717  OE1 GLN   94      23.805  26.879  44.946  1.00 13.60
ATOM    718  NE2 GLN   94      23.719  28.527  43.449  1.00  7.98
ATOM    719  N   GLU   95      20.396  30.531  47.820  1.00 11.78
ATOM    720  CA  GLU   95      19.974  31.899  47.643  1.00 13.47
ATOM    721  C   GLU   95      21.149  32.804  47.398  1.00 18.42
ATOM    722  O   GLU   95      22.206  32.623  47.985  1.00 19.23
ATOM    723  CB  GLU   95      19.277  32.427  48.878  1.00 13.52
ATOM    724  CG  GLU   95      18.009  31.684  49.215  1.00 28.46
ATOM    725  CD  GLU   95      17.657  32.016  50.622  1.00 45.93
ATOM    726  OE1 GLU   95      17.574  33.166  51.011  1.00100.00
ATOM    727  OE2 GLU   95      17.764  30.987  51.423  1.00 61.33
ATOM    728  N   ARG   96      20.929  33.838  46.601  1.00 16.81
ATOM    729  CA  ARG   96      21.978  34.783  46.342  1.00 16.87
ATOM    730  C   ARG   96      21.510  36.195  46.206  1.00 15.84
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FIG 5-12

ATOM	731	O	ARG	96	20.389	36.488	45.806	1.00	15.01
ATOM	732	CB	ARG	96	22.582	34.463	44.967	1.00	16.29
ATOM	733	CG	ARG	96	23.495	33.247	44.929	1.00	17.61
ATOM	734	CD	ARG	96	24.615	33.453	43.908	1.00	9.06
ATOM	735	NE	ARG	96	25.411	32.277	43.766	1.00	9.88
ATOM	736	CZ	ARG	96	25.434	31.493	42.693	1.00	20.03
ATOM	737	NH1	ARG	96	24.684	31.709	41.615	1.00	15.29
ATOM	738	NH2	ARG	96	26.236	30.430	42.714	1.00	11.03
ATOM	739	N	THR	97	22.470	37.068	46.344	1.00	13.39
ATOM	740	CA	THR	97	22.368	38.424	45.935	1.00	13.12
ATOM	741	C	THR	97	23.593	38.688	45.084	1.00	16.81
ATOM	742	O	THR	97	24.686	38.347	45.485	1.00	19.25
ATOM	743	CB	THR	97	22.282	39.442	47.066	1.00	26.27
ATOM	744	OG1	THR	97	21.225	39.101	47.945	1.00	31.43
ATOM	745	CG2	THR	97	22.038	40.804	46.445	1.00	15.90
ATOM	746	N	ILE	98	23.396	39.219	43.899	1.00	16.23
ATOM	747	CA	ILE	98	24.486	39.526	42.977	1.00	16.70
ATOM	748	C	ILE	98	24.533	41.017	42.686	1.00	21.10
ATOM	749	O	ILE	98	23.628	41.566	42.075	1.00	14.58
ATOM	750	CB	ILE	98	24.385	38.752	41.660	1.00	13.47
ATOM	751	CG1	ILE	98	24.480	37.236	41.890	1.00	16.09
ATOM	752	CG2	ILE	98	25.457	39.231	40.679	1.00	13.30
ATOM	753	CD1	ILE	98	23.875	36.431	40.738	1.00	13.93
ATOM	754	N	PHE	99	25.613	41.678	43.110	1.00	14.86
ATOM	755	CA	PHE	99	25.719	43.098	42.896	1.00	12.44
ATOM	756	C	PHE	99	26.514	43.441	41.699	1.00	20.37
ATOM	757	O	PHE	99	27.696	43.164	41.700	1.00	20.07
ATOM	758	CB	PHE	99	26.401	43.770	44.084	1.00	15.96
ATOM	759	CG	PHE	99	25.638	43.624	45.356	1.00	21.41
ATOM	760	CD1	PHE	99	25.863	42.524	46.189	1.00	24.98
ATOM	761	CD2	PHE	99	24.698	44.585	45.743	1.00	22.94
ATOM	762	CE1	PHE	99	25.176	42.400	47.400	1.00	32.06
ATOM	763	CE2	PHE	99	23.992	44.469	46.946	1.00	24.26
ATOM	764	CZ	PHE	99	24.235	43.369	47.771	1.00	28.19
ATOM	765	N	PHE	100	25.906	44.085	40.704	1.00	12.53
ATOM	766	CA	PHE	100	26.679	44.522	39.554	1.00	8.75
ATOM	767	C	PHE	100	27.294	45.855	39.872	1.00	21.81
ATOM	768	O	PHE	100	26.599	46.775	40.308	1.00	20.31
ATOM	769	CB	PHE	100	25.927	44.572	38.226	1.00	5.94
ATOM	770	CG	PHE	100	25.537	43.183	37.764	1.00	12.75
ATOM	771	CD1	PHE	100	24.426	42.538	38.325	1.00	16.31
ATOM	772	CD2	PHE	100	26.317	42.484	36.843	1.00	15.27
ATOM	773	CE1	PHE	100	24.087	41.230	37.975	1.00	13.50
ATOM	774	CE2	PHE	100	25.965	41.192	36.435	1.00	21.25
ATOM	775	CZ	PHE	100	24.852	40.567	37.014	1.00	21.06
ATOM	776	N	LYS	101	28.603	45.946	39.737	1.00	15.49
ATOM	777	CA	LYS	101	29.270	47.179	40.085	1.00	17.93
ATOM	778	C	LYS	101	28.732	48.349	39.287	1.00	13.71
ATOM	779	O	LYS	101	28.658	48.304	38.072	1.00	17.18
ATOM	780	CB	LYS	101	30.784	47.069	39.950	1.00	17.13
ATOM	781	CG	LYS	101	31.518	48.252	40.551	1.00	18.01
ATOM	782	CD	LYS	101	33.036	48.060	40.534	1.00	26.70
ATOM	783	CE	LYS	101	33.797	49.116	41.332	1.00	41.58
ATOM	784	N	ASP	102	28.353	49.403	39.997	1.00	18.09
ATOM	785	CA	ASP	102	27.805	50.618	39.368	1.00	23.08
ATOM	786	C	ASP	102	26.559	50.356	38.549	1.00	25.42
ATOM	787	O	ASP	102	26.292	51.061	37.586	1.00	23.34
ATOM	788	CB	ASP	102	28.840	51.369	38.516	1.00	26.27
ATOM	789	CG	ASP	102	30.109	51.629	39.296	1.00	57.01
ATOM	790	OD1	ASP	102	31.206	51.233	38.931	1.00	63.33
ATOM	791	OD2	ASP	102	29.886	52.200	40.464	1.00	47.66
ATOM	792	N	ASP	103	25.813	49.328	38.933	1.00	20.17
ATOM	793	CA	ASP	103	24.602	48.949	38.233	1.00	15.70
ATOM	794	C	ASP	103	23.608	48.284	39.189	1.00	18.47
ATOM	795	O	ASP	103	23.749	48.431	40.409	1.00	17.72
ATOM	796	CB	ASP	103	24.899	48.085	36.995	1.00	19.89
ATOM	797	CG	ASP	103	23.946	48.387	35.860	1.00	23.93

FIG 5-13

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ATOM    798  OD1 ASP   103      24.238  48.274  34.688  1.00 19.05
ATOM    799  OD2 ASP   103      22.774  48.809  36.283  1.00 23.89
ATOM    800  N   GLY   104      22.612  47.542  38.646  1.00 20.17
ATOM    801  CA  GLY   104      21.598  46.900  39.498  1.00 20.22
ATOM    802  C   GLY   104      22.055  45.619  40.180  1.00 24.68
ATOM    803  O   GLY   104      23.202  45.211  40.085  1.00 18.06
ATOM    804  N   ASN   105      21.125  44.967  40.872  1.00 15.71
ATOM    805  CA  ASN   105      21.425  43.703  41.510  1.00  8.89
ATOM    806  C   ASN   105      20.399  42.620  41.181  1.00 21.85
ATOM    807  O   ASN   105      19.255  42.911  40.824  1.00 15.17
ATOM    808  CB  ASN   105      21.605  43.840  43.001  1.00  8.58
ATOM    809  CG  ASN   105      20.359  44.366  43.697  1.00 43.57
ATOM    810  OD1 ASN   105      19.565  43.601  44.259  1.00 36.67
ATOM    811  ND2 ASN   105      20.178  45.674  43.659  1.00 36.47
ATOM    812  N   TYR   106      20.826  41.365  41.328  1.00 16.80
ATOM    813  CA  TYR   106      19.966  40.219  41.156  1.00 13.90
ATOM    814  C   TYR   106      19.763  39.543  42.475  1.00 11.05
ATOM    815  O   TYR   106      20.678  39.404  43.281  1.00 13.86
ATOM    816  CB  TYR   106      20.547  39.128  40.246  1.00 15.88
ATOM    817  CG  TYR   106      20.619  39.398  38.793  1.00 15.57
ATOM    818  CD1 TYR   106      19.952  40.458  38.178  1.00 13.14
ATOM    819  CD2 TYR   106      21.273  38.524  38.006  1.00 13.35
ATOM    820  CE1 TYR   106      20.038  40.632  36.793  1.00 13.44
ATOM    821  CE2 TYR   106      21.481  38.692  36.628  1.00 10.87
ATOM    822  CZ  TYR   106      20.814  39.751  36.025  1.00 15.93
ATOM    823  OH  TYR   106      20.970  39.931  34.670  1.00 17.32
ATOM    824  N   LYS   107      18.538  39.115  42.709  1.00 12.39
ATOM    825  CA  LYS   107      18.194  38.349  43.897  1.00 11.51
ATOM    826  C   LYS   107      17.619  37.037  43.397  1.00 17.25
ATOM    827  O   LYS   107      16.704  37.010  42.562  1.00 13.14
ATOM    828  CB  LYS   107      17.217  39.063  44.823  1.00 14.82
ATOM    829  CG  LYS   107      17.860  39.631  46.060  1.00 40.71
ATOM    830  CD  LYS   107      18.528  40.974  45.793  1.00 43.48
ATOM    831  N   THR   108      18.205  35.951  43.835  1.00 14.95
ATOM    832  CA  THR   108      17.774  34.658  43.352  1.00 11.97
ATOM    833  C   THR   108      17.463  33.696  44.468  1.00 15.81
ATOM    834  O   THR   108      18.043  33.734  45.582  1.00 18.68
ATOM    835  CB  THR   108      18.847  34.034  42.410  1.00 23.81
ATOM    836  OG1 THR   108      20.064  33.791  43.137  1.00 13.88
ATOM    837  CG2 THR   108      19.123  34.968  41.264  1.00 13.04
ATOM    838  N   ARG   109      16.560  32.804  44.154  1.00 13.57
ATOM    839  CA  ARG   109      16.212  31.751  45.048  1.00 12.56
ATOM    840  C   ARG   109      15.939  30.498  44.254  1.00 13.07
ATOM    841  O   ARG   109      15.239  30.509  43.249  1.00 12.52
ATOM    842  CB  ARG   109      15.069  32.100  45.959  1.00 17.32
ATOM    843  CG  ARG   109      14.767  30.995  46.932  1.00 17.92
ATOM    844  CD  ARG   109      13.400  31.160  47.610  1.00 19.99
ATOM    845  NE  ARG   109      12.821  29.854  47.883  1.00 36.05
ATOM    846  CZ  ARG   109      12.968  29.244  49.035  1.00 55.71
ATOM    847  NH1 ARG   109      13.630  29.815  50.046  1.00 44.11
ATOM    848  NH2 ARG   109      12.432  28.041  49.195  1.00 94.34
ATOM    849  N   ALA   110      16.577  29.414  44.635  1.00 13.26
ATOM    850  CA  ALA   110      16.377  28.207  43.870  1.00 12.68
ATOM    851  C   ALA   110      16.346  26.979  44.734  1.00 13.15
ATOM    852  O   ALA   110      16.829  26.965  45.869  1.00 16.75
ATOM    853  CB  ALA   110      17.465  28.059  42.822  1.00 17.31
ATOM    854  N   GLU   111      15.770  25.939  44.176  1.00 15.39
ATOM    855  CA  GLU   111      15.741  24.655  44.823  1.00 15.24
ATOM    856  C   GLU   111      16.438  23.678  43.926  1.00 12.08
ATOM    857  O   GLU   111      16.086  23.545  42.771  1.00 15.70
ATOM    858  CB  GLU   111      14.303  24.123  44.993  1.00 19.20
ATOM    859  CG  GLU   111      13.744  24.242  46.399  1.00 38.62
ATOM    860  CD  GLU   111      12.247  24.280  46.372  1.00 60.99
ATOM    861  OE1 GLU   111      11.589  23.843  45.432  1.00 76.05
ATOM    862  OE2 GLU   111      11.742  24.956  47.280  1.00 54.87
ATOM    863  N   VAL   112      17.438  22.965  44.457  1.00 10.72
ATOM    864  CA  VAL   112      18.063  21.978  43.631  1.00 10.98
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FIG 5-14

```
ATOM    865  C   VAL 112      17.968  20.630  44.261  1.00  8.62
ATOM    866  O   VAL 112      18.271  20.438  45.432  1.00 15.63
ATOM    867  CB  VAL 112      19.428  22.358  43.012  1.00 22.75
ATOM    868  CG1 VAL 112      19.966  23.704  43.487  1.00 16.69
ATOM    869  CG2 VAL 112      20.452  21.232  43.078  1.00 18.47
ATOM    870  N   LYS 113      17.415  19.732  43.516  1.00 14.67
ATOM    871  CA  LYS 113      17.175  18.421  44.045  1.00 16.41
ATOM    872  C   LYS 113      16.822  17.485  42.931  1.00  7.11
ATOM    873  O   LYS 113      16.695  17.893  41.808  1.00 16.27
ATOM    874  CB  LYS 113      16.032  18.497  45.036  1.00 22.50
ATOM    875  CG  LYS 113      14.792  19.084  44.376  1.00 20.40
ATOM    876  CD  LYS 113      13.509  18.321  44.703  1.00 44.65
ATOM    877  CE  LYS 113      12.526  19.134  45.528  1.00 54.02
ATOM    878  NZ  LYS 113      12.379  20.518  45.036  1.00100.00
ATOM    879  N   PHE 114      16.683  16.208  43.267  1.00 10.09
ATOM    880  CA  PHE 114      16.325  15.175  42.317  1.00 11.41
ATOM    881  C   PHE 114      14.806  14.975  42.181  1.00 14.18
ATOM    882  O   PHE 114      14.110  14.878  43.160  1.00 15.03
ATOM    883  CB  PHE 114      16.866  13.838  42.838  1.00 12.89
ATOM    884  CG  PHE 114      18.231  13.536  42.338  1.00 16.80
ATOM    885  CD1 PHE 114      19.344  13.795  43.139  1.00 18.61
ATOM    886  CD2 PHE 114      18.403  13.009  41.056  1.00 19.50
ATOM    887  CE1 PHE 114      20.627  13.500  42.665  1.00 22.78
ATOM    888  CE2 PHE 114      19.673  12.708  40.572  1.00 25.36
ATOM    889  CZ  PHE 114      20.780  12.953  41.387  1.00 23.99
ATOM    890  N   GLU 115      14.354  14.819  40.966  1.00 15.29
ATOM    891  CA  GLU 115      12.978  14.473  40.642  1.00 11.40
ATOM    892  C   GLU 115      13.121  13.193  39.906  1.00 13.30
ATOM    893  O   GLU 115      13.434  13.207  38.730  1.00 18.72
ATOM    894  CB  GLU 115      12.348  15.481  39.667  1.00  9.68
ATOM    895  CG  GLU 115      11.856  16.747  40.376  1.00 19.54
ATOM    896  CD  GLU 115      10.742  16.460  41.342  1.00 38.12
ATOM    897  OE1 GLU 115      10.181  15.395  41.431  1.00 34.84
ATOM    898  OE2 GLU 115      10.460  17.461  42.079  1.00 27.88
ATOM    899  N   GLY 116      13.005  12.087  40.585  1.00 14.51
ATOM    900  CA  GLY 116      13.225  10.861  39.869  1.00 15.91
ATOM    901  C   GLY 116      14.727  10.767  39.641  1.00 23.59
ATOM    902  O   GLY 116      15.516  10.922  40.570  1.00 19.35
ATOM    903  N   ASP 117      15.137  10.564  38.439  1.00 20.26
ATOM    904  CA  ASP 117      16.572  10.462  38.233  1.00 28.00
ATOM    905  C   ASP 117      17.237  11.677  37.598  1.00 22.39
ATOM    906  O   ASP 117      18.423  11.672  37.265  1.00 21.38
ATOM    907  CB  ASP 117      17.055   9.074  37.733  1.00 33.06
ATOM    908  CG  ASP 117      16.624   8.677  36.348  1.00 55.04
ATOM    909  OD1 ASP 117      16.230   9.468  35.495  1.00 59.57
ATOM    910  OD2 ASP 117      16.805   7.391  36.130  1.00 82.48
ATOM    911  N   THR 118      16.463  12.729  37.493  1.00 19.62
ATOM    912  CA  THR 118      16.889  13.981  36.910  1.00 18.21
ATOM    913  C   THR 118      17.186  14.988  37.976  1.00 18.92
ATOM    914  O   THR 118      16.498  15.064  38.996  1.00 15.94
ATOM    915  CB  THR 118      15.806  14.497  35.952  1.00 19.03
ATOM    916  OG1 THR 118      15.552  13.508  34.990  1.00 21.42
ATOM    917  CG2 THR 118      16.217  15.793  35.275  1.00 15.49
ATOM    918  N   LEU 119      18.284  15.681  37.805  1.00 13.66
ATOM    919  CA  LEU 119      18.679  16.706  38.759  1.00 13.50
ATOM    920  C   LEU 119      18.036  17.992  38.269  1.00  8.81
ATOM    921  O   LEU 119      18.194  18.368  37.091  1.00 12.49
ATOM    922  CB  LEU 119      20.243  16.815  38.839  1.00 12.25
ATOM    923  CG  LEU 119      20.845  17.678  39.951  1.00  3.90
ATOM    924  CD1 LEU 119      20.701  19.167  39.669  1.00 10.11
ATOM    925  CD2 LEU 119      20.366  17.311  41.333  1.00  7.36
ATOM    926  N   VAL 120      17.230  18.595  39.170  1.00 13.34
ATOM    927  CA  VAL 120      16.466  19.797  38.859  1.00 13.77
ATOM    928  C   VAL 120      16.929  21.039  39.587  1.00  8.56
ATOM    929  O   VAL 120      17.135  21.039  40.762  1.00 13.32
ATOM    930  CB  VAL 120      14.939  19.566  39.082  1.00 17.50
ATOM    931  CG1 VAL 120      14.133  20.790  38.642  1.00 17.58
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FIG 5-15

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ATOM    932  CG2 VAL   120      14.501  18.351  38.246  1.00 15.35
ATOM    933  N   ASN   121      17.067  22.111  38.839  1.00 12.24
ATOM    934  CA  ASN   121      17.424  23.405  39.400  1.00 11.78
ATOM    935  C   ASN   121      16.301  24.382  39.060  1.00 11.18
ATOM    936  O   ASN   121      16.195  24.802  37.934  1.00 11.09
ATOM    937  CB  ASN   121      18.753  23.928  38.791  1.00 11.41
ATOM    938  CG  ASN   121      19.201  25.261  39.367  1.00 11.07
ATOM    939  OD1 ASN   121      18.773  25.654  40.461  1.00 12.06
ATOM    940  ND2 ASN   121      20.124  25.938  38.670  1.00 11.90
ATOM    941  N   ARG   122      15.470  24.706  40.029  1.00 13.69
ATOM    942  CA  ARG   122      14.348  25.610  39.825  1.00 12.99
ATOM    943  C   ARG   122      14.622  26.946  40.498  1.00  5.89
ATOM    944  O   ARG   122      14.749  27.011  41.723  1.00 14.47
ATOM    945  CB  ARG   122      13.068  25.025  40.417  1.00 15.99
ATOM    946  CG  ARG   122      12.478  23.921  39.589  1.00 30.23
ATOM    947  CD  ARG   122      11.282  23.244  40.281  1.00 60.61
ATOM    948  N   ILE   123      14.663  27.992  39.680  1.00 11.46
ATOM    949  CA  ILE   123      15.030  29.340  40.095  1.00 11.86
ATOM    950  C   ILE   123      13.991  30.450  39.835  1.00 10.54
ATOM    951  O   ILE   123      13.370  30.535  38.765  1.00 12.83
ATOM    952  CB  ILE   123      16.296  29.757  39.292  1.00 15.41
ATOM    953  CG1 ILE   123      17.316  28.585  39.180  1.00 12.27
ATOM    954  CG2 ILE   123      16.944  30.993  39.918  1.00 14.01
ATOM    955  CD1 ILE   123      17.652  28.242  37.743  1.00  7.74
ATOM    956  N   GLU   124      13.953  31.358  40.793  1.00 11.36
ATOM    957  CA  GLU   124      13.189  32.572  40.700  1.00 15.20
ATOM    958  C   GLU   124      14.168  33.713  40.811  1.00 11.93
ATOM    959  O   GLU   124      14.919  33.797  41.780  1.00 15.61
ATOM    960  CB  GLU   124      12.028  32.677  41.751  1.00 19.74
ATOM    961  CG  GLU   124      12.387  33.337  43.089  1.00 72.94
ATOM    962  N   LEU   125      14.183  34.550  39.808  1.00 12.19
ATOM    963  CA  LEU   125      15.092  35.654  39.767  1.00 15.00
ATOM    964  C   LEU   125      14.420  37.011  39.722  1.00 19.35
ATOM    965  O   LEU   125      13.563  37.267  38.893  1.00 18.41
ATOM    966  CB  LEU   125      15.976  35.533  38.510  1.00 14.29
ATOM    967  CG  LEU   125      17.003  36.683  38.375  1.00 17.65
ATOM    968  CD1 LEU   125      18.302  36.083  37.849  1.00 13.46
ATOM    969  CD2 LEU   125      16.511  37.732  37.367  1.00 12.09
ATOM    970  N   LYS   126      14.890  37.897  40.554  1.00 12.73
ATOM    971  CA  LYS   126      14.391  39.260  40.579  1.00 15.92
ATOM    972  C   LYS   126      15.563  40.276  40.445  1.00 18.53
ATOM    973  O   LYS   126      16.489  40.246  41.246  1.00 19.86
ATOM    974  CB  LYS   126      13.611  39.487  41.877  1.00 17.31
ATOM    975  CG  LYS   126      12.853  40.786  41.923  1.00 33.94
ATOM    976  CD  LYS   126      11.366  40.601  41.675  1.00 60.87
ATOM    977  CE  LYS   126      10.652  41.929  41.521  1.00 52.70
ATOM    978  NZ  LYS   126      11.229  42.988  42.367  1.00 47.22
ATOM    979  N   GLY   127      15.514  41.127  39.411  1.00 18.71
ATOM    980  CA  GLY   127      16.551  42.151  39.121  1.00 17.32
ATOM    981  C   GLY   127      16.012  43.572  39.272  1.00 25.32
ATOM    982  O   GLY   127      14.981  43.908  38.693  1.00 20.14
ATOM    983  N   ILE   128      16.706  44.404  40.070  1.00 18.42
ATOM    984  CA  ILE   128      16.282  45.787  40.243  1.00 21.04
ATOM    985  C   ILE   128      17.405  46.789  40.196  1.00 25.93
ATOM    986  O   ILE   128      18.562  46.496  40.429  1.00 19.37
ATOM    987  CB  ILE   128      15.482  46.052  41.504  1.00 23.82
ATOM    988  CG1 ILE   128      16.408  45.888  42.701  1.00 23.86
ATOM    989  CG2 ILE   128      14.272  45.120  41.577  1.00 28.95
ATOM    990  CD1 ILE   128      15.824  46.391  44.013  1.00 29.89
ATOM    991  N   ASP   129      16.999  48.002  39.918  1.00 20.26
ATOM    992  CA  ASP   129      17.861  49.124  39.882  1.00 18.53
ATOM    993  C   ASP   129      18.864  49.086  38.801  1.00 20.36
ATOM    994  O   ASP   129      19.949  49.632  38.953  1.00 24.28
ATOM    995  CB  ASP   129      18.498  49.407  41.253  1.00 20.57
ATOM    996  CG  ASP   129      17.545  50.077  42.226  1.00 43.70
ATOM    997  OD1 ASP   129      16.653  50.842  41.883  1.00 49.42
ATOM    998  OD2 ASP   129      17.770  49.740  43.475  1.00 38.07
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FIG 5-16

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ATOM    999  N   PHE   130      18.510  48.493  37.693  1.00 16.40
ATOM   1000  CA  PHE   130      19.433  48.459  36.563  1.00 16.99
ATOM   1001  C   PHE   130      19.330  49.732  35.756  1.00 35.37
ATOM   1002  O   PHE   130      18.242  50.318  35.623  1.00 27.34
ATOM   1003  CB  PHE   130      19.248  47.223  35.657  1.00 18.07
ATOM   1004  CG  PHE   130      19.809  45.980  36.312  1.00 19.10
ATOM   1005  CD1 PHE   130      19.021  45.210  37.171  1.00 16.15
ATOM   1006  CD2 PHE   130      21.126  45.572  36.073  1.00 19.17
ATOM   1007  CE1 PHE   130      19.536  44.074  37.801  1.00 23.37
ATOM   1008  CE2 PHE   130      21.665  44.445  36.703  1.00 21.11
ATOM   1009  CZ  PHE   130      20.867  43.703  37.575  1.00 22.13
ATOM   1010  N   LYS   131      20.464  50.169  35.218  1.00 31.09
ATOM   1011  CA  LYS   131      20.477  51.371  34.400  1.00 27.52
ATOM   1012  C   LYS   131      20.105  51.045  32.992  1.00 25.57
ATOM   1013  O   LYS   131      20.695  50.169  32.343  1.00 22.97
ATOM   1014  CB  LYS   131      21.796  52.109  34.438  1.00 32.64
ATOM   1015  CG  LYS   131      22.153  52.633  35.813  1.00 38.34
ATOM   1016  CD  LYS   131      23.646  52.886  35.975  1.00 75.76
ATOM   1017  N   GLU   132      19.116  51.751  32.509  1.00 26.88
ATOM   1018  CA  GLU   132      18.623  51.484  31.189  1.00 28.42
ATOM   1019  C   GLU   132      19.710  51.514  30.140  1.00 36.19
ATOM   1020  O   GLU   132      19.617  50.862  29.101  1.00 39.24
ATOM   1021  CB  GLU   132      17.374  52.331  30.830  1.00 29.04
ATOM   1022  N   ASP   133      20.752  52.254  30.438  1.00 40.08
ATOM   1023  CA  ASP   133      21.883  52.442  29.525  1.00 45.36
ATOM   1024  C   ASP   133      23.224  51.861  30.049  1.00 50.61
ATOM   1025  O   ASP   133      24.299  52.243  29.572  1.00 52.14
ATOM   1026  CB  ASP   133      22.063  53.946  29.332  1.00 50.45
ATOM   1027  CG  ASP   133      22.109  54.642  30.670  1.00 87.10
ATOM   1028  OD1 ASP   133      21.408  54.314  31.624  1.00 91.27
ATOM   1029  OD2 ASP   133      23.047  55.552  30.739  1.00100.00
ATOM   1030  N   GLY   134      23.159  50.970  31.053  1.00 37.06
ATOM   1031  CA  GLY   134      24.349  50.375  31.639  1.00 30.22
ATOM   1032  C   GLY   134      24.845  49.228  30.803  1.00 23.10
ATOM   1033  O   GLY   134      24.360  48.990  29.685  1.00 19.23
ATOM   1034  N   ASN   135      25.807  48.486  31.341  1.00 18.66
ATOM   1035  CA  ASN   135      26.339  47.370  30.563  1.00 18.03
ATOM   1036  C   ASN   135      25.372  46.199  30.406  1.00 15.75
ATOM   1037  O   ASN   135      25.485  45.430  29.461  1.00 16.03
ATOM   1038  CB  ASN   135      27.665  46.883  31.139  1.00 19.27
ATOM   1039  CG  ASN   135      28.743  47.943  31.108  1.00 20.99
ATOM   1040  OD1 ASN   135      28.969  48.595  30.078  1.00 25.69
ATOM   1041  ND2 ASN   135      29.423  48.095  32.239  1.00 22.57
ATOM   1042  N   ILE   136      24.444  46.052  31.362  1.00 18.14
ATOM   1043  CA  ILE   136      23.494  44.924  31.368  1.00 19.78
ATOM   1044  C   ILE   136      22.331  45.086  30.384  1.00 23.76
ATOM   1045  O   ILE   136      22.178  44.313  29.395  1.00 22.53
ATOM   1046  CB  ILE   136      23.078  44.500  32.804  1.00 21.24
ATOM   1047  CG1 ILE   136      24.230  43.728  33.423  1.00 28.44
ATOM   1048  CG2 ILE   136      21.899  43.543  32.770  1.00 22.77
ATOM   1049  CD1 ILE   136      25.346  44.596  33.935  1.00 12.39
ATOM   1050  N   LEU   137      21.543  46.117  30.640  1.00 18.21
ATOM   1051  CA  LEU   137      20.394  46.415  29.815  1.00 23.30
ATOM   1052  C   LEU   137      20.828  46.875  28.470  1.00 27.26
ATOM   1053  O   LEU   137      20.181  46.619  27.488  1.00 27.00
ATOM   1054  CB  LEU   137      19.442  47.430  30.490  1.00 21.74
ATOM   1055  CG  LEU   137      18.828  46.852  31.762  1.00 22.56
ATOM   1056  CD1 LEU   137      17.856  47.837  32.415  1.00 22.27
ATOM   1057  CD2 LEU   137      18.119  45.554  31.424  1.00 37.52
ATOM   1058  N   GLY   138      21.979  47.527  28.432  1.00 22.14
ATOM   1059  CA  GLY   138      22.510  48.033  27.187  1.00 20.03
ATOM   1060  C   GLY   138      23.157  46.959  26.368  1.00 20.16
ATOM   1061  O   GLY   138      23.600  47.202  25.264  1.00 22.44
ATOM   1062  N   HIS   139      23.246  45.756  26.903  1.00 18.27
ATOM   1063  CA  HIS   139      23.859  44.688  26.148  1.00 20.24
ATOM   1064  C   HIS   139      25.301  44.939  25.616  1.00 20.13
ATOM   1065  O   HIS   139      25.605  44.745  24.439  1.00 17.97
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FIG 5-17

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ATOM   1066  CB  HIS   139      22.931  44.207  25.018  1.00 22.20
ATOM   1067  CG  HIS   139      21.708  43.551  25.550  1.00 25.52
ATOM   1068  ND1 HIS   139      21.666  42.182  25.785  1.00 25.67
ATOM   1069  CD2 HIS   139      20.525  44.092  25.927  1.00 28.09
ATOM   1070  CE1 HIS   139      20.474  41.918  26.275  1.00 27.50
ATOM   1071  NE2 HIS   139      19.766  43.044  26.382  1.00 29.53
ATOM   1072  N   LYS   140      26.187  45.311  26.525  1.00 23.51
ATOM   1073  CA  LYS   140      27.569  45.638  26.197  1.00 25.82
ATOM   1074  C   LYS   140      28.600  44.537  26.560  1.00 26.28
ATOM   1075  O   LYS   140      29.824  44.730  26.391  1.00 22.29
ATOM   1076  CB  LYS   140      27.977  46.937  26.911  1.00 27.56
ATOM   1077  CG  LYS   140      27.269  48.217  26.445  1.00 31.19
ATOM   1078  CD  LYS   140      27.234  49.254  27.582  1.00 51.32
ATOM   1079  CE  LYS   140      26.924  50.696  27.169  1.00 47.92
ATOM   1080  NZ  LYS   140      27.112  51.663  28.284  1.00 73.76
ATOM   1081  N   LEU   141      28.116  43.403  27.115  1.00 19.33
ATOM   1082  CA  LEU   141      28.987  42.296  27.559  1.00 14.32
ATOM   1083  C   LEU   141      29.366  41.401  26.427  1.00 20.75
ATOM   1084  O   LEU   141      28.526  41.087  25.620  1.00 19.01
ATOM   1085  CB  LEU   141      28.313  41.488  28.676  1.00 12.53
ATOM   1086  CG  LEU   141      27.979  42.352  29.875  1.00 17.54
ATOM   1087  CD1 LEU   141      27.700  41.469  31.070  1.00 24.81
ATOM   1088  CD2 LEU   141      29.116  43.310  30.182  1.00 27.50
ATOM   1089  N   GLU   142      30.644  40.987  26.346  1.00 14.76
ATOM   1090  CA  GLU   142      31.040  40.059  25.311  1.00 13.43
ATOM   1091  C   GLU   142      30.462  38.651  25.641  1.00 15.69
ATOM   1092  O   GLU   142      30.175  38.393  26.787  1.00 16.43
ATOM   1093  CB  GLU   142      32.558  39.866  25.204  1.00 14.73
ATOM   1094  CG  GLU   142      33.290  41.077  24.624  1.00 29.30
ATOM   1095  CD  GLU   142      34.787  41.003  24.825  1.00 56.32
ATOM   1096  OE1 GLU   142      35.340  40.098  25.420  1.00 31.70
ATOM   1097  OE2 GLU   142      35.430  42.015  24.321  1.00 34.10
ATOM   1098  N   TYR   143      30.365  37.873  24.632  1.00 16.30
ATOM   1099  CA  TYR   143      29.837  36.542  24.764  1.00 20.04
ATOM   1100  C   TYR   143      30.925  35.559  25.049  1.00 12.46
ATOM   1101  O   TYR   143      31.327  34.792  24.193  1.00 16.99
ATOM   1102  CB  TYR   143      29.035  36.118  23.498  1.00 20.96
ATOM   1103  CG  TYR   143      28.187  34.857  23.674  1.00 16.12
ATOM   1104  CD1 TYR   143      27.040  34.859  24.472  1.00 18.24
ATOM   1105  CD2 TYR   143      28.512  33.684  22.986  1.00 12.87
ATOM   1106  CE1 TYR   143      26.257  33.708  24.615  1.00 17.91
ATOM   1107  CE2 TYR   143      27.735  32.530  23.104  1.00 16.58
ATOM   1108  CZ  TYR   143      26.603  32.551  23.914  1.00 17.35
ATOM   1109  OH  TYR   143      25.861  31.432  24.035  1.00 23.40
ATOM   1110  N   ASN   144      31.392  35.597  26.251  1.00 12.40
ATOM   1111  CA  ASN   144      32.428  34.703  26.689  1.00 12.05
ATOM   1112  C   ASN   144      32.433  34.675  28.193  1.00 15.75
ATOM   1113  O   ASN   144      31.637  35.369  28.837  1.00 14.58
ATOM   1114  CB  ASN   144      33.823  35.038  26.068  1.00 18.45
ATOM   1115  CG  ASN   144      34.310  36.445  26.374  1.00 18.98
ATOM   1116  OD1 ASN   144      34.150  36.951  27.488  1.00 20.34
ATOM   1117  ND2 ASN   144      34.891  37.085  25.382  1.00 23.02
ATOM   1118  N   TYR   145      33.311  33.876  28.773  1.00 12.16
ATOM   1119  CA  TYR   145      33.343  33.765  30.195  1.00 10.63
ATOM   1120  C   TYR   145      34.765  33.458  30.730  1.00 14.58
ATOM   1121  O   TYR   145      35.510  32.751  30.090  1.00 19.83
ATOM   1122  CB  TYR   145      32.404  32.627  30.571  1.00  9.76
ATOM   1123  CG  TYR   145      31.698  32.916  31.826  1.00 11.86
ATOM   1124  CD1 TYR   145      30.515  33.658  31.808  1.00  9.04
ATOM   1125  CD2 TYR   145      32.188  32.419  33.030  1.00 10.07
ATOM   1126  CE1 TYR   145      29.860  33.948  32.999  1.00  8.36
ATOM   1127  CE2 TYR   145      31.544  32.707  34.235  1.00 15.32
ATOM   1128  CZ  TYR   145      30.375  33.469  34.206  1.00 11.69
ATOM   1129  OH  TYR   145      29.730  33.735  35.376  1.00 15.23
ATOM   1130  N   ASN   146      35.086  33.931  31.923  1.00 15.36
ATOM   1131  CA  ASN   146      36.415  33.737  32.560  1.00 17.00
ATOM   1132  C   ASN   146      36.426  32.612  33.539  1.00 19.68
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FIG 5-18

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ATOM   1133  O   ASN  146      35.395  32.043  33.848  1.00 14.71
ATOM   1134  CB  ASN  146      36.844  35.062  33.235  1.00 11.89
ATOM   1135  CG  ASN  146      37.013  36.147  32.215  1.00 35.45
ATOM   1136  OD1 ASN  146      37.533  35.890  31.105  1.00 31.63
ATOM   1137  ND2 ASN  146      36.547  37.349  32.553  1.00 19.74
ATOM   1138  N   SER  147      37.630  32.338  34.201  1.00 12.09
ATOM   1139  CA  SER  147      37.804  31.320  35.266  1.00  8.55
ATOM   1140  C   SER  147      37.769  31.999  36.575  1.00 11.70
ATOM   1141  O   SER  147      38.219  33.125  36.671  1.00 16.56
ATOM   1142  CB  SER  147      39.148  30.540  35.129  1.00  9.87
ATOM   1143  OG  SER  147      39.212  29.980  33.828  1.00 33.20
ATOM   1144  N   HIS  148      37.195  31.365  37.583  1.00  5.53
ATOM   1145  CA  HIS  148      37.090  31.998  38.850  1.00  8.06
ATOM   1146  C   HIS  148      37.346  31.038  39.949  1.00 11.30
ATOM   1147  O   HIS  148      37.328  29.844  39.754  1.00 16.87
ATOM   1148  CB  HIS  148      35.648  32.608  39.067  1.00 11.29
ATOM   1149  CG  HIS  148      35.215  33.554  37.972  1.00 10.84
ATOM   1150  ND1 HIS  148      34.548  33.121  36.836  1.00 12.77
ATOM   1151  CD2 HIS  148      35.403  34.887  37.851  1.00  8.82
ATOM   1152  CE1 HIS  148      34.389  34.178  36.060  1.00  8.84
ATOM   1153  NE2 HIS  148      34.882  35.242  36.647  1.00  8.82
ATOM   1154  N   ASN  149      37.534  31.579  41.125  1.00 10.80
ATOM   1155  CA  ASN  149      37.626  30.805  42.345  1.00 13.35
ATOM   1156  C   ASN  149      36.409  31.157  43.205  1.00 14.47
ATOM   1157  O   ASN  149      36.099  32.320  43.387  1.00 18.17
ATOM   1158  CB  ASN  149      38.890  31.093  43.184  1.00 12.67
ATOM   1159  CG  ASN  149      40.148  30.822  42.424  1.00 20.21
ATOM   1160  OD1 ASN  149      40.993  31.713  42.281  1.00 56.34
ATOM   1161  ND2 ASN  149      40.210  29.641  41.818  1.00 16.44
ATOM   1162  N   VAL  150      35.773  30.144  43.741  1.00 14.65
ATOM   1163  CA  VAL  150      34.588  30.262  44.552  1.00 12.92
ATOM   1164  C   VAL  150      34.910  29.806  45.943  1.00 16.30
ATOM   1165  O   VAL  150      35.257  28.665  46.147  1.00 17.83
ATOM   1166  CB  VAL  150      33.482  29.382  43.914  1.00 15.22
ATOM   1167  CG1 VAL  150      32.252  29.297  44.765  1.00 14.09
ATOM   1168  CG2 VAL  150      33.172  29.791  42.464  1.00 10.94
ATOM   1169  N   TYR  151      34.796  30.716  46.900  1.00 17.64
ATOM   1170  CA  TYR  151      35.139  30.440  48.275  1.00 18.31
ATOM   1171  C   TYR  151      34.003  29.917  49.117  1.00 24.35
ATOM   1172  O   TYR  151      32.963  30.536  49.239  1.00 20.83
ATOM   1173  CB  TYR  151      35.793  31.681  48.920  1.00 20.15
ATOM   1174  CG  TYR  151      37.025  32.033  48.141  1.00 25.86
ATOM   1175  CD1 TYR  151      37.003  32.989  47.127  1.00 26.00
ATOM   1176  CD2 TYR  151      38.200  31.315  48.355  1.00 28.66
ATOM   1177  CE1 TYR  151      38.151  33.234  46.369  1.00 33.73
ATOM   1178  CE2 TYR  151      39.360  31.550  47.619  1.00 29.01
ATOM   1179  CZ  TYR  151      39.325  32.512  46.618  1.00 29.55
ATOM   1180  OH  TYR  151      40.449  32.737  45.877  1.00 38.69
ATOM   1181  N   ILE  152      34.250  28.791  49.753  1.00 17.71
ATOM   1182  CA  ILE  152      33.255  28.159  50.572  1.00 14.12
ATOM   1183  C   ILE  152      33.619  28.056  52.000  1.00 18.51
ATOM   1184  O   ILE  152      34.728  27.703  52.336  1.00 22.05
ATOM   1185  CB  ILE  152      32.979  26.776  50.060  1.00 16.66
ATOM   1186  CG1 ILE  152      32.431  26.875  48.638  1.00 11.30
ATOM   1187  CG2 ILE  152      32.017  26.078  51.021  1.00 17.96
ATOM   1188  CD1 ILE  152      32.377  25.559  47.949  1.00 13.48
ATOM   1189  N   MSE  153      32.623  28.278  52.841  1.00 17.41
ATOM   1190  CA  MSE  153      32.789  28.162  54.269  1.00 22.61
ATOM   1191  C   MSE  153      31.534  27.648  54.916  1.00 27.31
ATOM   1192  O   MSE  153      30.433  27.831  54.396  1.00 20.50
ATOM   1193  CB  MSE  153      33.145  29.490  54.855  1.00 19.11
ATOM   1194  CG  MSE  153      34.010  30.302  53.957  1.00100.00
ATOM   1195  SE  MSE  153      34.060  32.117  54.524  1.00100.00
ATOM   1196  CE  MSE  153      33.463  31.798  56.330  1.00 30.27
ATOM   1197  N   ALA  154      31.733  26.983  56.053  1.00 22.29
ATOM   1198  CA  ALA  154      30.669  26.389  56.796  1.00 22.66
ATOM   1199  C   ALA  154      29.820  27.401  57.552  1.00 29.00
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FIG 5-19

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ATOM   1200  O   ALA  154      30.274  28.457  57.960  1.00 27.02
ATOM   1201  CB  ALA  154      31.224  25.336  57.744  1.00 19.78
ATOM   1202  N   ASP  155      28.566  27.063  57.726  1.00 29.48
ATOM   1203  CA  ASP  155      27.669  27.887  58.484  1.00 32.18
ATOM   1204  C   ASP  155      26.976  27.019  59.511  1.00 44.51
ATOM   1205  O   ASP  155      25.898  26.492  59.274  1.00 39.55
ATOM   1206  CB  ASP  155      26.659  28.617  57.597  1.00 31.70
ATOM   1207  CG  ASP  155      26.140  29.851  58.247  1.00 49.89
ATOM   1208  OD1 ASP  155      26.595  30.297  59.277  1.00 46.67
ATOM   1209  OD2 ASP  155      25.187  30.422  57.565  1.00 76.07
ATOM   1210  N   LYS  156      27.646  26.816  60.629  1.00 46.37
ATOM   1211  CA  LYS  156      27.116  25.954  61.654  1.00 53.23
ATOM   1212  C   LYS  156      25.750  26.369  62.224  1.00 65.62
ATOM   1213  O   LYS  156      25.012  25.520  62.703  1.00 65.54
ATOM   1214  CB  LYS  156      28.147  25.612  62.725  1.00 59.51
ATOM   1215  N   GLN  157      25.398  27.655  62.138  1.00 68.32
ATOM   1216  CA  GLN  157      24.119  28.135  62.670  1.00 73.00
ATOM   1217  C   GLN  157      22.891  27.767  61.817  1.00 87.53
ATOM   1218  O   GLN  157      21.778  27.547  62.325  1.00 96.16
ATOM   1219  N   LYS  158      23.095  27.725  60.506  1.00 72.49
ATOM   1220  CA  LYS  158      22.040  27.386  59.593  1.00 66.19
ATOM   1221  C   LYS  158      22.235  25.985  59.040  1.00 58.21
ATOM   1222  O   LYS  158      21.447  25.524  58.226  1.00 59.85
ATOM   1223  N   ASN  159      23.303  25.294  59.502  1.00 40.00
ATOM   1224  CA  ASN  159      23.582  23.944  59.012  1.00 36.67
ATOM   1225  C   ASN  159      23.755  24.002  57.500  1.00 34.11
ATOM   1226  O   ASN  159      23.223  23.167  56.754  1.00 31.69
ATOM   1227  CB  ASN  159      22.431  22.952  59.367  1.00 46.42
ATOM   1228  CG  ASN  159      22.842  21.485  59.428  1.00 80.46
ATOM   1229  OD1 ASN  159      23.850  21.121  60.054  1.00100.00
ATOM   1230  ND2 ASN  159      22.003  20.620  58.854  1.00 58.09
ATOM   1231  N   GLY  160      24.474  25.044  57.062  1.00 22.34
ATOM   1232  CA  GLY  160      24.686  25.247  55.663  1.00 17.58
ATOM   1233  C   GLY  160      26.055  25.791  55.433  1.00 26.75
ATOM   1234  O   GLY  160      26.960  25.664  56.271  1.00 25.57
ATOM   1235  N   ILE  161      26.200  26.395  54.277  1.00 23.28
ATOM   1236  CA  ILE  161      27.442  26.975  53.909  1.00 16.45
ATOM   1237  C   ILE  161      27.200  28.354  53.395  1.00 15.77
ATOM   1238  O   ILE  161      26.118  28.680  52.962  1.00 15.95
ATOM   1239  CB  ILE  161      28.129  26.117  52.864  1.00 19.27
ATOM   1240  CG1 ILE  161      27.237  26.016  51.619  1.00 18.53
ATOM   1241  CG2 ILE  161      28.351  24.735  53.445  1.00 21.96
ATOM   1242  CD1 ILE  161      28.009  25.614  50.350  1.00 14.44
ATOM   1243  N   LYS  162      28.226  29.169  53.471  1.00 17.96
ATOM   1244  CA  LYS  162      28.187  30.508  52.948  1.00 14.42
ATOM   1245  C   LYS  162      29.216  30.524  51.857  1.00 17.73
ATOM   1246  O   LYS  162      30.249  29.875  51.991  1.00 19.16
ATOM   1247  CB  LYS  162      28.480  31.540  54.055  1.00 18.15
ATOM   1248  CG  LYS  162      27.221  31.963  54.796  1.00 42.08
ATOM   1249  CD  LYS  162      27.493  32.787  56.039  1.00 70.42
ATOM   1250  N   VAL  163      28.911  31.176  50.759  1.00 13.74
ATOM   1251  CA  VAL  163      29.798  31.201  49.629  1.00 11.95
ATOM   1252  C   VAL  163      29.928  32.610  49.103  1.00 19.30
ATOM   1253  O   VAL  163      28.944  33.318  48.983  1.00 19.84
ATOM   1254  CB  VAL  163      29.249  30.268  48.532  1.00 15.89
ATOM   1255  CG1 VAL  163      30.105  30.277  47.261  1.00 12.09
ATOM   1256  CG2 VAL  163      29.029  28.852  49.077  1.00 15.86
ATOM   1257  N   ASN  164      31.146  32.999  48.733  1.00 14.03
ATOM   1258  CA  ASN  164      31.382  34.310  48.195  1.00 15.58
ATOM   1259  C   ASN  164      32.396  34.271  47.050  1.00 20.08
ATOM   1260  O   ASN  164      33.268  33.386  46.988  1.00 23.49
ATOM   1261  CB  ASN  164      31.732  35.325  49.308  1.00 20.32
ATOM   1262  CG  ASN  164      33.196  35.697  49.330  1.00 89.21
ATOM   1263  OD1 ASN  164      34.020  34.987  49.929  1.00100.00
ATOM   1264  ND2 ASN  164      33.515  36.831  48.700  1.00 91.46
ATOM   1265  N   PHE  165      32.244  35.207  46.109  1.00 17.37
ATOM   1266  CA  PHE  165      33.133  35.301  44.953  1.00 10.86
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FIG 5-20

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ATOM   1267  C   PHE  165      32.751  36.445  44.071  1.00 15.53
ATOM   1268  O   PHE  165      31.686  37.020  44.251  1.00 17.16
ATOM   1269  CB  PHE  165      33.207  33.960  44.187  1.00 12.86
ATOM   1270  CG  PHE  165      31.862  33.486  43.622  1.00 14.35
ATOM   1271  CD1 PHE  165      31.510  33.749  42.293  1.00 14.61
ATOM   1272  CD2 PHE  165      30.978  32.757  44.413  1.00 13.56
ATOM   1273  CE1 PHE  165      30.300  33.297  41.759  1.00 22.67
ATOM   1274  CE2 PHE  165      29.774  32.282  43.893  1.00 15.76
ATOM   1275  CZ  PHE  165      29.426  32.572  42.573  1.00 16.20
ATOM   1276  N   LYS  166      33.641  36.799  43.132  1.00 10.79
ATOM   1277  CA  LYS  166      33.417  37.864  42.162  1.00 10.74
ATOM   1278  C   LYS  166      33.603  37.344  40.774  1.00 15.95
ATOM   1279  O   LYS  166      34.602  36.727  40.470  1.00 22.80
ATOM   1280  CB  LYS  166      34.387  39.055  42.249  1.00 16.61
ATOM   1281  CG  LYS  166      34.573  39.688  43.573  1.00 18.11
ATOM   1282  CD  LYS  166      35.540  40.875  43.454  1.00 32.56
ATOM   1283  CE  LYS  166      35.272  41.966  44.476  1.00 48.19
ATOM   1284  NZ  LYS  166      34.823  41.435  45.782  1.00 85.81
ATOM   1285  N   ILE  167      32.703  37.704  39.911  1.00  9.75
ATOM   1286  CA  ILE  167      32.768  37.340  38.558  1.00  9.35
ATOM   1287  C   ILE  167      33.203  38.542  37.823  1.00 14.36
ATOM   1288  O   ILE  167      32.811  39.640  38.170  1.00 16.22
ATOM   1289  CB  ILE  167      31.379  36.929  38.005  1.00 13.16
ATOM   1290  CG1 ILE  167      30.909  35.624  38.669  1.00 13.02
ATOM   1291  CG2 ILE  167      31.423  36.726  36.472  1.00  7.91
ATOM   1292  CD1 ILE  167      31.773  34.415  38.344  1.00 19.57
ATOM   1293  N   ARG  168      34.005  38.299  36.815  1.00 12.19
ATOM   1294  CA  ARG  168      34.500  39.308  35.945  1.00 15.07
ATOM   1295  C   ARG  168      33.948  39.122  34.528  1.00 16.64
ATOM   1296  O   ARG  168      34.278  38.156  33.836  1.00 17.70
ATOM   1297  CB  ARG  168      36.024  39.287  35.944  1.00 16.54
ATOM   1298  CG  ARG  168      36.580  39.632  37.321  1.00 25.54
ATOM   1299  CD  ARG  168      37.894  38.910  37.601  1.00 63.52
ATOM   1300  NE  ARG  168      38.380  38.191  36.416  1.00 73.52
ATOM   1301  CZ  ARG  168      38.764  36.926  36.416  1.00 67.92
ATOM   1302  NH1 ARG  168      38.795  36.192  37.527  1.00 57.44
ATOM   1303  NH2 ARG  168      39.192  36.375  35.271  1.00 59.15
ATOM   1304  N   HIS  169      33.090  40.064  34.098  1.00 14.88
ATOM   1305  CA  HIS  169      32.505  40.025  32.758  1.00 13.24
ATOM   1306  C   HIS  169      33.214  41.001  31.839  1.00 12.64
ATOM   1307  O   HIS  169      33.306  42.203  32.121  1.00 14.99
ATOM   1308  CB  HIS  169      30.970  40.374  32.760  1.00 10.46
ATOM   1309  CG  HIS  169      30.097  39.474  33.573  1.00  6.54
ATOM   1310  ND1 HIS  169      29.724  38.246  33.111  1.00 12.63
ATOM   1311  CD2 HIS  169      29.474  39.695  34.764  1.00 10.21
ATOM   1312  CE1 HIS  169      28.892  37.718  34.031  1.00 10.53
ATOM   1313  NE2 HIS  169      28.734  38.566  35.063  1.00 11.84
ATOM   1314  N   ASN  170      33.691  40.513  30.737  1.00 10.66
ATOM   1315  CA  ASN  170      34.349  41.368  29.812  1.00 15.87
ATOM   1316  C   ASN  170      33.356  42.224  29.067  1.00 25.06
ATOM   1317  O   ASN  170      32.386  41.701  28.537  1.00 16.60
ATOM   1318  CB  ASN  170      35.110  40.550  28.755  1.00 19.60
ATOM   1319  CG  ASN  170      36.245  39.717  29.312  1.00 18.70
ATOM   1320  OD1 ASN  170      36.702  38.752  28.684  1.00 48.29
ATOM   1321  ND2 ASN  170      36.695  40.073  30.480  1.00 19.13
ATOM   1322  N   ILE  171      33.662  43.527  28.947  1.00 18.75
ATOM   1323  CA  ILE  171      32.848  44.460  28.168  1.00 16.74
ATOM   1324  C   ILE  171      33.459  44.638  26.791  1.00 19.51
ATOM   1325  O   ILE  171      34.643  44.595  26.642  1.00 21.06
ATOM   1326  CB  ILE  171      32.713  45.804  28.842  1.00 20.46
ATOM   1327  CG1 ILE  171      32.089  45.617  30.193  1.00 24.79
ATOM   1328  CG2 ILE  171      31.852  46.727  27.997  1.00 19.03
ATOM   1329  CD1 ILE  171      32.630  46.599  31.229  1.00 41.65
ATOM   1330  N   GLU  172      32.632  44.818  25.804  1.00 16.54
ATOM   1331  CA  GLU  172      33.034  44.933  24.420  1.00 17.00
ATOM   1332  C   GLU  172      34.110  45.987  24.147  1.00 26.80
ATOM   1333  O   GLU  172      34.776  45.898  23.125  1.00 29.20
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FIG 5-21

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ATOM   1334  CB  GLU   172      31.813  45.165  23.509  1.00 22.46
ATOM   1335  CG  GLU   172      31.122  46.531  23.786  1.00 58.53
ATOM   1336  CD  GLU   172      29.871  46.783  22.933  1.00100.00
ATOM   1337  OE1 GLU   172      29.415  45.970  22.156  1.00100.00
ATOM   1338  OE2 GLU   172      29.370  47.982  23.149  1.00100.00
ATOM   1339  N   ASP   173      34.277  46.934  25.034  1.00 24.41
ATOM   1340  CA  ASP   173      35.292  47.978  24.852  1.00 25.03
ATOM   1341  C   ASP   173      36.651  47.624  25.455  1.00 33.40
ATOM   1342  O   ASP   173      37.561  48.451  25.518  1.00 30.42
ATOM   1343  CB  ASP   173      34.822  49.319  25.401  1.00 23.30
ATOM   1344  CG  ASP   173      34.743  49.358  26.912  1.00 32.47
ATOM   1345  OD1 ASP   173      34.406  50.355  27.513  1.00 37.58
ATOM   1346  OD2 ASP   173      34.949  48.196  27.504  1.00 49.22
ATOM   1347  N   GLY   174      36.766  46.410  25.956  1.00 23.87
ATOM   1348  CA  GLY   174      38.019  45.994  26.537  1.00 21.30
ATOM   1349  C   GLY   174      38.012  46.090  28.044  1.00 19.99
ATOM   1350  O   GLY   174      38.927  45.585  28.709  1.00 20.45
ATOM   1351  N   SER   175      36.972  46.767  28.598  1.00 13.88
ATOM   1352  CA  SER   175      36.898  46.931  30.034  1.00  8.70
ATOM   1353  C   SER   175      36.296  45.728  30.765  1.00 17.30
ATOM   1354  O   SER   175      36.136  44.655  30.175  1.00 18.77
ATOM   1355  CB  SER   175      36.288  48.235  30.450  1.00 14.07
ATOM   1356  OG  SER   175      36.360  48.316  31.865  1.00 24.79
ATOM   1357  N   VAL   176      35.963  45.912  32.051  1.00 13.74
ATOM   1358  CA  VAL   176      35.415  44.826  32.864  1.00 16.46
ATOM   1359  C   VAL   176      34.191  45.204  33.703  1.00 22.46
ATOM   1360  O   VAL   176      34.159  46.254  34.334  1.00 21.31
ATOM   1361  CB  VAL   176      36.477  44.285  33.818  1.00 24.43
ATOM   1362  CG1 VAL   176      35.847  43.344  34.827  1.00 27.45
ATOM   1363  CG2 VAL   176      37.532  43.536  33.035  1.00 25.65
ATOM   1364  N   GLN   177      33.234  44.269  33.787  1.00 15.47
ATOM   1365  CA  GLN   177      32.048  44.430  34.647  1.00 15.40
ATOM   1366  C   GLN   177      32.102  43.457  35.813  1.00 10.60
ATOM   1367  O   GLN   177      32.027  42.243  35.634  1.00 13.65
ATOM   1368  CB  GLN   177      30.709  44.283  33.872  1.00 15.57
ATOM   1369  CG  GLN   177      29.468  44.294  34.828  1.00 19.13
ATOM   1370  CD  GLN   177      29.108  45.678  35.361  1.00 14.91
ATOM   1371  OE1 GLN   177      28.759  46.588  34.574  1.00 20.17
ATOM   1372  NE2 GLN   177      29.128  45.821  36.690  1.00 17.28
ATOM   1373  N   LEU   178      32.227  43.993  37.018  1.00  8.17
ATOM   1374  CA  LEU   178      32.313  43.180  38.181  1.00 16.66
ATOM   1375  C   LEU   178      30.954  42.786  38.712  1.00 20.93
ATOM   1376  O   LEU   178      30.033  43.608  38.753  1.00 14.66
ATOM   1377  CB  LEU   178      33.089  43.896  39.293  1.00 20.63
ATOM   1378  CG  LEU   178      34.286  43.110  39.815  1.00 39.28
ATOM   1379  CD1 LEU   178      33.831  42.087  40.852  1.00 45.14
ATOM   1380  CD2 LEU   178      35.018  42.426  38.648  1.00 39.52
ATOM   1381  N   ALA   179      30.869  41.550  39.171  1.00 16.72
ATOM   1382  CA  ALA   179      29.652  41.033  39.754  1.00 15.55
ATOM   1383  C   ALA   179      29.932  40.277  41.040  1.00 15.70
ATOM   1384  O   ALA   179      30.337  39.119  41.028  1.00 15.91
ATOM   1385  CB  ALA   179      28.853  40.197  38.731  1.00 14.08
ATOM   1386  N   ASP   180      29.694  40.946  42.155  1.00  8.88
ATOM   1387  CA  ASP   180      29.897  40.407  43.480  1.00  7.18
ATOM   1388  C   ASP   180      28.802  39.460  43.891  1.00 17.07
ATOM   1389  O   ASP   180      27.651  39.844  43.987  1.00 18.22
ATOM   1390  CB  ASP   180      29.934  41.509  44.509  1.00 13.06
ATOM   1391  CG  ASP   180      31.285  41.902  44.935  1.00 46.28
ATOM   1392  OD1 ASP   180      31.981  41.206  45.655  1.00 60.46
ATOM   1393  OD2 ASP   180      31.574  43.121  44.560  1.00 46.61
ATOM   1394  N   HIS   181      29.173  38.242  44.197  1.00 14.51
ATOM   1395  CA  HIS   181      28.213  37.223  44.575  1.00 10.49
ATOM   1396  C   HIS   181      28.218  36.897  46.049  1.00 14.28
ATOM   1397  O   HIS   181      29.255  36.580  46.607  1.00 17.40
ATOM   1398  CB  HIS   181      28.450  35.915  43.769  1.00  9.89
ATOM   1399  CG  HIS   181      28.077  35.972  42.328  1.00 10.38
ATOM   1400  ND1 HIS   181      28.606  36.926  41.455  1.00 12.24
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FIG 5-22

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ATOM   1401  CD2 HIS   181      27.279  35.146  41.606  1.00 10.42
ATOM   1402  CE1 HIS   181      28.093  36.678  40.269  1.00  9.97
ATOM   1403  NE2 HIS   181      27.314  35.594  40.316  1.00  9.38
ATOM   1404  N   TYR   182      27.029  36.897  46.668  1.00 10.40
ATOM   1405  CA  TYR   182      26.848  36.518  48.062  1.00 13.86
ATOM   1406  C   TYR   182      25.871  35.393  48.089  1.00 20.61
ATOM   1407  O   TYR   182      24.819  35.520  47.532  1.00 16.35
ATOM   1408  CB  TYR   182      26.359  37.664  48.934  1.00 21.12
ATOM   1409  CG  TYR   182      27.421  38.693  49.062  1.00 34.16
ATOM   1410  CD1 TYR   182      27.521  39.715  48.120  1.00 46.06
ATOM   1411  CD2 TYR   182      28.389  38.616  50.064  1.00 38.56
ATOM   1412  CE1 TYR   182      28.532  40.674  48.197  1.00 57.53
ATOM   1413  CE2 TYR   182      29.418  39.559  50.147  1.00 40.76
ATOM   1414  CZ  TYR   182      29.480  40.594  49.216  1.00 54.61
ATOM   1415  OH  TYR   182      30.461  41.534  49.308  1.00 61.92
ATOM   1416  N   GLN   183      26.246  34.277  48.686  1.00 17.63
ATOM   1417  CA  GLN   183      25.410  33.104  48.583  1.00 16.37
ATOM   1418  C   GLN   183      25.289  32.311  49.863  1.00 21.39
ATOM   1419  O   GLN   183      26.260  32.174  50.623  1.00 19.86
ATOM   1420  CB  GLN   183      25.984  32.219  47.422  1.00 13.33
ATOM   1421  CG  GLN   183      25.651  30.688  47.457  1.00 17.38
ATOM   1422  CD  GLN   183      26.411  29.884  46.389  1.00 17.27
ATOM   1423  OE1 GLN   183      26.975  30.454  45.456  1.00 13.80
ATOM   1424  NE2 GLN   183      26.361  28.553  46.473  1.00 13.94
ATOM   1425  N   GLN   184      24.080  31.739  50.055  1.00 19.74
ATOM   1426  CA  GLN   184      23.760  30.829  51.168  1.00 16.55
ATOM   1427  C   GLN   184      23.033  29.582  50.658  1.00 13.60
ATOM   1428  O   GLN   184      22.219  29.640  49.747  1.00 18.01
ATOM   1429  CB  GLN   184      22.949  31.444  52.330  1.00 20.11
ATOM   1430  CG  GLN   184      23.364  32.855  52.768  1.00 74.84
ATOM   1431  CD  GLN   184      22.312  33.517  53.657  1.00100.00
ATOM   1432  OE1 GLN   184      21.159  33.054  53.752  1.00 97.99
ATOM   1433  NE2 GLN   184      22.689  34.625  54.286  1.00100.00
ATOM   1434  N   ASN   185      23.418  28.446  51.207  1.00 14.76
ATOM   1435  CA  ASN   185      22.831  27.155  50.887  1.00 13.86
ATOM   1436  C   ASN   185      22.421  26.463  52.166  1.00 16.06
ATOM   1437  O   ASN   185      23.176  26.402  53.172  1.00 17.39
ATOM   1438  CB  ASN   185      23.761  26.212  50.119  1.00 15.20
ATOM   1439  CG  ASN   185      24.110  26.696  48.748  1.00 12.75
ATOM   1440  OD1 ASN   185      24.704  27.758  48.592  1.00 22.56
ATOM   1441  ND2 ASN   185      23.830  25.868  47.763  1.00 17.70
ATOM   1442  N   THR   186      21.227  25.941  52.139  1.00 18.01
ATOM   1443  CA  THR   186      20.707  25.227  53.288  1.00 17.40
ATOM   1444  C   THR   186      19.976  24.010  52.824  1.00 23.63
ATOM   1445  O   THR   186      19.389  23.991  51.730  1.00 24.57
ATOM   1446  CB  THR   186      19.856  26.100  54.206  1.00 28.82
ATOM   1447  OG1 THR   186      18.874  26.752  53.446  1.00 35.65
ATOM   1448  CG2 THR   186      20.753  27.121  54.903  1.00 28.86
ATOM   1449  N   PRO   187      20.101  22.951  53.620  1.00 22.40
ATOM   1450  CA  PRO   187      19.504  21.683  53.269  1.00 20.28
ATOM   1451  C   PRO   187      17.988  21.757  53.288  1.00 22.41
ATOM   1452  O   PRO   187      17.390  22.518  54.071  1.00 25.07
ATOM   1453  CB  PRO   187      19.977  20.682  54.337  1.00 19.79
ATOM   1454  CG  PRO   187      20.840  21.449  55.338  1.00 26.98
ATOM   1455  CD  PRO   187      20.786  22.918  54.949  1.00 22.04
ATOM   1456  N   ILE   188      17.382  20.957  52.453  1.00 18.77
ATOM   1457  CA  ILE   188      15.907  20.855  52.407  1.00 20.12
ATOM   1458  C   ILE   188      15.470  19.766  53.389  1.00 31.58
ATOM   1459  O   ILE   188      14.596  19.966  54.202  1.00 38.58
ATOM   1460  CB  ILE   188      15.385  20.574  50.991  1.00 21.52
ATOM   1461  CG1 ILE   188      15.555  21.775  50.102  1.00 16.10
ATOM   1462  CG2 ILE   188      13.916  20.141  50.981  1.00 28.85
ATOM   1463  CD1 ILE   188      15.139  21.471  48.660  1.00 15.31
ATOM   1464  N   GLY   189      16.142  18.618  53.352  1.00 32.39
ATOM   1465  CA  GLY   189      15.833  17.531  54.283  1.00 32.94
ATOM   1466  C   GLY   189      16.339  17.817  55.702  1.00 40.20
ATOM   1467  O   GLY   189      17.016  18.810  55.967  1.00 35.57
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FIG 5-23

ATOM	1468	N	ASP	190	16.003	16.928	56.617	1.00	49.41
ATOM	1469	CA	ASP	190	16.392	17.047	58.021	1.00	55.01
ATOM	1470	C	ASP	190	17.556	16.115	58.338	1.00	56.16
ATOM	1471	O	ASP	190	18.083	16.100	59.463	1.00	58.30
ATOM	1472	CB	ASP	190	15.195	16.734	58.955	1.00	63.89
ATOM	1473	CG	ASP	190	14.592	15.365	58.686	1.00	99.67
ATOM	1474	OD1	ASP	190	14.599	14.466	59.514	1.00	100.00
ATOM	1475	OD2	ASP	190	14.088	15.240	57.470	1.00	100.00
ATOM	1476	N	GLY	191	17.921	15.312	57.323	1.00	47.20
ATOM	1477	CA	GLY	191	19.015	14.347	57.419	1.00	44.96
ATOM	1478	C	GLY	191	20.359	15.044	57.587	1.00	34.43
ATOM	1479	O	GLY	191	20.452	16.266	57.438	1.00	29.96
ATOM	1480	N	PRO	192	21.402	14.264	57.905	1.00	27.26
ATOM	1481	CA	PRO	192	22.737	14.834	58.100	1.00	24.01
ATOM	1482	C	PRO	192	23.444	15.274	56.787	1.00	20.55
ATOM	1483	O	PRO	192	23.323	14.648	55.740	1.00	23.84
ATOM	1484	CB	PRO	192	23.583	13.764	58.825	1.00	21.00
ATOM	1485	CG	PRO	192	22.739	12.501	58.915	1.00	27.49
ATOM	1486	CD	PRO	192	21.330	12.863	58.448	1.00	27.26
ATOM	1487	N	VAL	193	24.193	16.363	56.892	1.00	17.87
ATOM	1488	CA	VAL	193	24.964	16.902	55.792	1.00	19.51
ATOM	1489	C	VAL	193	26.380	17.108	56.249	1.00	22.37
ATOM	1490	O	VAL	193	26.663	17.189	57.443	1.00	23.84
ATOM	1491	CB	VAL	193	24.449	18.245	55.256	1.00	25.24
ATOM	1492	CG1	VAL	193	23.059	18.118	54.632	1.00	21.90
ATOM	1493	CG2	VAL	193	24.497	19.322	56.346	1.00	24.81
ATOM	1494	N	LEU	194	27.253	17.241	55.277	1.00	19.04
ATOM	1495	CA	LEU	194	28.654	17.438	55.516	1.00	20.29
ATOM	1496	C	LEU	194	29.006	18.930	55.571	1.00	18.71
ATOM	1497	O	LEU	194	28.907	19.615	54.591	1.00	20.13
ATOM	1498	CB	LEU	194	29.412	16.806	54.327	1.00	22.92
ATOM	1499	CG	LEU	194	29.994	15.423	54.542	1.00	30.60
ATOM	1500	CD1	LEU	194	29.227	14.642	55.595	1.00	35.19
ATOM	1501	CD2	LEU	194	30.048	14.672	53.211	1.00	25.61
ATOM	1502	N	LEU	195	29.453	19.430	56.713	1.00	17.39
ATOM	1503	CA	LEU	195	29.881	20.808	56.785	1.00	18.83
ATOM	1504	C	LEU	195	31.389	20.837	56.579	1.00	28.32
ATOM	1505	O	LEU	195	32.161	20.152	57.281	1.00	21.98
ATOM	1506	CB	LEU	195	29.489	21.525	58.072	1.00	22.20
ATOM	1507	CG	LEU	195	28.055	21.349	58.444	1.00	26.40
ATOM	1508	CD1	LEU	195	27.937	21.508	59.941	1.00	31.99
ATOM	1509	CD2	LEU	195	27.225	22.395	57.726	1.00	26.90
ATOM	1510	N	PRO	196	31.789	21.610	55.597	1.00	21.58
ATOM	1511	CA	PRO	196	33.177	21.666	55.154	1.00	22.17
ATOM	1512	C	PRO	196	34.080	22.623	55.892	1.00	29.56
ATOM	1513	O	PRO	196	33.635	23.588	56.490	1.00	29.04
ATOM	1514	CB	PRO	196	33.054	22.265	53.752	1.00	22.77
ATOM	1515	CG	PRO	196	31.761	23.104	53.735	1.00	18.99
ATOM	1516	CD	PRO	196	30.910	22.567	54.861	1.00	16.42
ATOM	1517	N	ASP	197	35.379	22.410	55.716	1.00	22.95
ATOM	1518	CA	ASP	197	36.364	23.370	56.134	1.00	19.71
ATOM	1519	C	ASP	197	36.556	24.295	54.931	1.00	24.74
ATOM	1520	O	ASP	197	36.251	23.913	53.800	1.00	24.88
ATOM	1521	CB	ASP	197	37.711	22.730	56.446	1.00	22.28
ATOM	1522	CG	ASP	197	37.690	21.913	57.687	1.00	43.93
ATOM	1523	OD1	ASP	197	36.912	22.117	58.608	1.00	53.47
ATOM	1524	OD2	ASP	197	38.634	21.006	57.694	1.00	31.58
ATOM	1525	N	ASN	198	37.062	25.501	55.168	1.00	19.74
ATOM	1526	CA	ASN	198	37.254	26.470	54.118	1.00	15.38
ATOM	1527	C	ASN	198	37.974	25.889	52.971	1.00	19.61
ATOM	1528	O	ASN	198	38.958	25.236	53.134	1.00	22.69
ATOM	1529	CB	ASN	198	38.013	27.704	54.614	1.00	24.48
ATOM	1530	CG	ASN	198	37.235	28.504	55.632	1.00	52.21
ATOM	1531	OD1	ASN	198	36.107	28.174	55.961	1.00	34.54
ATOM	1532	ND2	ASN	198	37.854	29.556	56.150	1.00	55.11
ATOM	1533	N	HIS	199	37.462	26.125	51.801	1.00	16.30
ATOM	1534	CA	HIS	199	38.071	25.627	50.616	1.00	15.30

FIG 5-24

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ATOM   1535  C   HIS   199      37.496  26.357  49.450  1.00 14.85
ATOM   1536  O   HIS   199      36.757  27.295  49.643  1.00 16.45
ATOM   1537  CB  HIS   199      37.988  24.103  50.471  1.00 16.53
ATOM   1538  CG  HIS   199      36.597  23.628  50.218  1.00 16.65
ATOM   1539  ND1 HIS   199      35.695  23.491  51.244  1.00 17.85
ATOM   1540  CD2 HIS   199      35.987  23.282  49.048  1.00 18.67
ATOM   1541  CE1 HIS   199      34.561  23.052  50.688  1.00 19.45
ATOM   1542  NE2 HIS   199      34.716  22.905  49.364  1.00 18.74
ATOM   1543  N   TYR   200      37.879  25.998  48.247  1.00 12.56
ATOM   1544  CA  TYR   200      37.334  26.689  47.100  1.00 14.01
ATOM   1545  C   TYR   200      37.207  25.824  45.870  1.00 15.57
ATOM   1546  O   TYR   200      37.793  24.751  45.768  1.00 20.20
ATOM   1547  CB  TYR   200      38.030  28.011  46.779  1.00 19.79
ATOM   1548  CG  TYR   200      39.382  27.745  46.202  1.00 22.25
ATOM   1549  CD1 TYR   200      39.543  27.526  44.835  1.00 22.53
ATOM   1550  CD2 TYR   200      40.473  27.605  47.057  1.00 25.73
ATOM   1551  CE1 TYR   200      40.800  27.222  44.317  1.00 35.51
ATOM   1552  CE2 TYR   200      41.739  27.314  46.559  1.00 29.34
ATOM   1553  CZ  TYR   200      41.896  27.132  45.186  1.00 54.14
ATOM   1554  OH  TYR   200      43.153  26.820  44.703  1.00 62.66
ATOM   1555  N   LEU   201      36.393  26.309  44.946  1.00 15.07
ATOM   1556  CA  LEU   201      36.147  25.680  43.678  1.00 11.01
ATOM   1557  C   LEU   201      36.753  26.532  42.593  1.00 17.30
ATOM   1558  O   LEU   201      36.619  27.753  42.610  1.00 20.19
ATOM   1559  CB  LEU   201      34.628  25.518  43.354  1.00 10.09
ATOM   1560  CG  LEU   201      33.749  25.027  44.480  1.00 13.41
ATOM   1561  CD1 LEU   201      32.293  24.938  43.954  1.00 17.11
ATOM   1562  CD2 LEU   201      34.196  23.635  44.927  1.00 23.03
ATOM   1563  N   SER   202      37.407  25.868  41.651  1.00 10.75
ATOM   1564  CA  SER   202      38.047  26.490  40.528  1.00  8.51
ATOM   1565  C   SER   202      37.222  26.189  39.294  1.00 11.56
ATOM   1566  O   SER   202      36.919  25.038  38.996  1.00 14.58
ATOM   1567  CB  SER   202      39.485  25.987  40.442  1.00 15.68
ATOM   1568  OG  SER   202      40.067  26.353  39.228  1.00 36.44
ATOM   1569  N   THR   203      36.798  27.241  38.601  1.00 12.36
ATOM   1570  CA  THR   203      35.879  27.067  37.499  1.00 15.60
ATOM   1571  C   THR   203      36.417  27.521  36.195  1.00 20.19
ATOM   1572  O   THR   203      37.192  28.472  36.114  1.00 18.29
ATOM   1573  CB  THR   203      34.565  27.892  37.757  1.00 20.51
ATOM   1574  OG1 THR   203      34.911  29.260  37.780  1.00 20.39
ATOM   1575  CG2 THR   203      33.935  27.557  39.093  1.00  6.80
ATOM   1576  N   GLN   204      35.913  26.883  35.164  1.00 10.30
ATOM   1577  CA  GLN   204      36.173  27.271  33.807  1.00 14.85
ATOM   1578  C   GLN   204      34.956  26.980  32.921  1.00 23.14
ATOM   1579  O   GLN   204      34.334  25.932  33.056  1.00 21.66
ATOM   1580  CB  GLN   204      37.475  26.696  33.237  1.00 20.33
ATOM   1581  CG  GLN   204      37.271  25.371  32.518  1.00 40.16
ATOM   1582  CD  GLN   204      38.588  24.722  32.193  1.00 59.76
ATOM   1583  OE1 GLN   204      39.011  24.716  31.035  1.00 41.80
ATOM   1584  NE2 GLN   204      39.276  24.241  33.235  1.00 34.80
ATOM   1585  N   SER   205      34.619  27.913  32.021  1.00 15.83
ATOM   1586  CA  SER   205      33.447  27.762  31.172  1.00 14.60
ATOM   1587  C   SER   205      33.654  28.307  29.783  1.00 20.21
ATOM   1588  O   SER   205      34.282  29.337  29.581  1.00 17.82
ATOM   1589  CB  SER   205      32.197  28.445  31.758  1.00 11.88
ATOM   1590  OG  SER   205      32.121  28.406  33.177  1.00 15.45
ATOM   1591  N   ALA   206      33.065  27.630  28.827  1.00 13.00
ATOM   1592  CA  ALA   206      33.079  28.029  27.426  1.00  9.99
ATOM   1593  C   ALA   206      31.623  28.192  26.924  1.00 21.23
ATOM   1594  O   ALA   206      30.809  27.306  27.139  1.00 14.10
ATOM   1595  CB  ALA   206      33.751  26.936  26.596  1.00 13.45
ATOM   1596  N   LEU   207      31.335  29.320  26.263  1.00 16.09
ATOM   1597  CA  LEU   207      30.036  29.617  25.706  1.00 12.07
ATOM   1598  C   LEU   207      30.070  29.445  24.235  1.00 19.76
ATOM   1599  O   LEU   207      31.014  29.840  23.576  1.00 20.82
ATOM   1600  CB  LEU   207      29.580  31.057  26.004  1.00  8.24
ATOM   1601  CG  LEU   207      29.744  31.493  27.457  1.00 16.35
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FIG 5-25

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ATOM   1602  CD1 LEU   207      28.955  32.790  27.707  1.00 13.78
ATOM   1603  CD2 LEU   207      29.268  30.406  28.400  1.00 18.79
ATOM   1604  N   SER   208      29.011  28.863  23.698  1.00 15.38
ATOM   1605  CA  SER   208      28.914  28.692  22.270  1.00 13.74
ATOM   1606  C   SER   208      27.449  28.852  21.794  1.00 20.16
ATOM   1607  O   SER   208      26.548  29.085  22.594  1.00 15.81
ATOM   1608  CB  SER   208      29.495  27.367  21.822  1.00 17.82
ATOM   1609  OG  SER   208      28.769  26.311  22.431  1.00 31.45
ATOM   1610  N   LYS   209      27.242  28.738  20.485  1.00 16.50
ATOM   1611  CA  LYS   209      25.907  28.828  19.906  1.00 18.02
ATOM   1612  C   LYS   209      25.637  27.610  19.031  1.00 29.99
ATOM   1613  O   LYS   209      26.578  27.004  18.502  1.00 32.55
ATOM   1614  CB  LYS   209      25.783  30.100  19.082  1.00 20.96
ATOM   1615  CG  LYS   209      24.746  31.055  19.606  1.00 34.50
ATOM   1616  CD  LYS   209      25.262  31.964  20.666  1.00 22.72
ATOM   1617  CE  LYS   209      24.370  33.159  20.896  1.00 18.96
ATOM   1618  NZ  LYS   209      23.565  33.067  22.116  1.00 27.39
ATOM   1619  N   ASP   210      24.347  27.241  18.912  1.00 27.01
ATOM   1620  CA  ASP   210      23.890  26.159  18.038  1.00 24.62
ATOM   1621  C   ASP   210      23.465  26.793  16.705  1.00 26.77
ATOM   1622  O   ASP   210      22.468  27.514  16.605  1.00 23.00
ATOM   1623  CB  ASP   210      22.744  25.361  18.691  1.00 24.43
ATOM   1624  CG  ASP   210      22.197  24.249  17.839  1.00 35.55
ATOM   1625  OD1 ASP   210      22.333  24.185  16.631  1.00 36.53
ATOM   1626  OD2 ASP   210      21.499  23.400  18.535  1.00 45.51
ATOM   1627  N   PRO   211      24.306  26.618  15.708  1.00 30.25
ATOM   1628  CA  PRO   211      24.120  27.224  14.397  1.00 30.30
ATOM   1629  C   PRO   211      22.733  26.982  13.770  1.00 39.72
ATOM   1630  O   PRO   211      22.253  27.782  12.959  1.00 37.65
ATOM   1631  CB  PRO   211      25.197  26.620  13.500  1.00 29.99
ATOM   1632  CG  PRO   211      25.782  25.418  14.255  1.00 38.59
ATOM   1633  CD  PRO   211      25.158  25.405  15.647  1.00 35.05
ATOM   1634  N   ASN   212      22.102  25.868  14.140  1.00 39.64
ATOM   1635  CA  ASN   212      20.808  25.515  13.592  1.00 39.60
ATOM   1636  C   ASN   212      19.642  25.894  14.497  1.00 41.92
ATOM   1637  O   ASN   212      18.485  25.518  14.263  1.00 42.30
ATOM   1638  CB  ASN   212      20.788  24.028  13.236  1.00 48.64
ATOM   1639  CG  ASN   212      21.883  23.678  12.230  1.00 53.61
ATOM   1640  N   GLU   213      19.947  26.675  15.520  1.00 27.84
ATOM   1641  CA  GLU   213      18.953  27.080  16.478  1.00 20.43
ATOM   1642  C   GLU   213      18.485  28.527  16.241  1.00 29.95
ATOM   1643  O   GLU   213      19.247  29.475  16.324  1.00 32.77
ATOM   1644  CB  GLU   213      19.535  26.878  17.894  1.00 16.45
ATOM   1645  CG  GLU   213      18.594  27.326  18.995  1.00 18.29
ATOM   1646  CD  GLU   213      17.229  26.703  18.853  1.00 38.01
ATOM   1647  OE1 GLU   213      16.238  27.334  18.508  1.00 25.07
ATOM   1648  OE2 GLU   213      17.223  25.423  19.122  1.00 19.17
ATOM   1649  N   LYS   214      17.223  28.713  15.963  1.00 22.99
ATOM   1650  CA  LYS   214      16.721  30.081  15.726  1.00 22.84
ATOM   1651  C   LYS   214      16.252  30.778  16.982  1.00 21.50
ATOM   1652  O   LYS   214      16.130  32.016  17.032  1.00 28.15
ATOM   1653  CB  LYS   214      15.653  30.197  14.606  1.00 27.58
ATOM   1654  CG  LYS   214      16.153  29.816  13.209  1.00 32.71
ATOM   1655  CD  LYS   214      16.752  30.979  12.431  1.00 55.31
ATOM   1656  N   ARG   215      15.947  30.028  13.014  1.00 14.52
ATOM   1657  CA  ARG   215      15.518  30.726  12.209  1.00 15.58
ATOM   1658  C   ARG   215      16.719  31.382  11.892  1.00 21.87
ATOM   1659  O   ARG   215      17.848  31.075  11.572  1.00 26.69
ATOM   1660  CB  ARG   215      14.808  29.804  10.159  1.00 18.82
ATOM   1661  CG  ARG   215      13.660  29.067  10.475  1.00 23.30
ATOM   1662  CD  ARG   215      13.220  27.806  10.205  1.00 15.45
ATOM   1663  NE  ARG   215      14.107  26.668  10.929  1.00 28.08
ATOM   1664  CZ  ARG   215      14.022  25.473  10.543  1.00 21.38
ATOM   1665  NH1 ARG   215      13.074  25.215  11.455  1.00 23.92
ATOM   1666  NH2 ARG   215      14.893  24.514  10.225  1.00 20.46
ATOM   1667  N   ASP   216      16.466  32.275  10.830  1.00 16.72
ATOM   1668  CA  ASP   216      17.556  32.895  11.617  1.00 19.06
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FIG 5-26

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ATOM   1669  C    ASP  216      18.047  31.817  22.607  1.00 20.02
ATOM   1670  O    ASP  216      17.261  31.214  23.350  1.00 18.45
ATOM   1671  CB   ASP  216      17.066  34.169  22.383  1.00 21.33
ATOM   1672  CG   ASP  216      18.138  35.140  22.893  1.00 20.97
ATOM   1673  OD1  ASP  216      17.869  36.079  23.620  1.00 28.46
ATOM   1674  OD2  ASP  216      19.342  34.900  22.441  1.00 20.37
ATOM   1675  N    HIS  217      19.332  31.537  22.589  1.00 13.18
ATOM   1676  CA   HIS  217      19.813  30.482  23.433  1.00 11.21
ATOM   1677  C    HIS  217      21.313  30.614  23.723  1.00 21.35
ATOM   1678  O    HIS  217      22.014  31.471  23.163  1.00 15.03
ATOM   1679  CB   HIS  217      19.587  29.168  22.690  1.00 13.03
ATOM   1680  CG   HIS  217      20.525  29.025  21.542  1.00 15.49
ATOM   1681  ND1  HIS  217      20.463  29.871  20.449  1.00 17.88
ATOM   1682  CD2  HIS  217      21.589  28.172  21.361  1.00 17.51
ATOM   1683  CE1  HIS  217      21.457  29.524  19.635  1.00 17.94
ATOM   1684  NE2  HIS  217      22.152  28.501  20.151  1.00 17.59
ATOM   1685  N    MSE  218      21.794  29.725  24.576  1.00 11.26
ATOM   1686  CA   MSE  218      23.186  29.642  24.887  1.00 11.49
ATOM   1687  C    MSE  218      23.560  28.198  25.094  1.00 24.15
ATOM   1688  O    MSE  218      22.822  27.446  25.751  1.00 20.70
ATOM   1689  CB   MSE  218      23.539  30.421  26.172  1.00 12.84
ATOM   1690  CG   MSE  218      24.809  30.004  26.907  1.00 12.59
ATOM   1691  SE   MSE  218      25.267  31.128  28.434  1.00 29.94
ATOM   1692  CE   MSE  218      24.039  30.502  29.781  1.00 13.54
ATOM   1693  N    VAL  219      24.727  27.824  24.558  1.00 15.62
ATOM   1694  CA   VAL  219      25.309  26.518  24.782  1.00 10.58
ATOM   1695  C    VAL  219      26.473  26.689  25.753  1.00 16.54
ATOM   1696  O    VAL  219      27.280  27.604  25.585  1.00 15.54
ATOM   1697  CB   VAL  219      25.774  25.883  23.498  1.00 15.08
ATOM   1698  CG1  VAL  219      26.330  24.495  23.824  1.00 14.34
ATOM   1699  CG2  VAL  219      24.599  25.766  22.512  1.00 15.78
ATOM   1700  N    LEU  220      26.523  25.836  26.783  1.00 10.95
ATOM   1701  CA   LEU  220      27.490  25.939  27.850  1.00 11.01
ATOM   1702  C    LEU  220      28.206  24.643  28.184  1.00 21.26
ATOM   1703  O    LEU  220      27.592  23.577  28.324  1.00 15.94
ATOM   1704  CB   LEU  220      26.807  26.545  29.100  1.00 13.75
ATOM   1705  CG   LEU  220      27.624  26.578  30.402  1.00 21.10
ATOM   1706  CD1  LEU  220      28.433  27.875  30.483  1.00 23.53
ATOM   1707  CD2  LEU  220      26.663  26.556  31.586  1.00 22.04
ATOM   1708  N    LEU  221      29.570  24.758  28.273  1.00 19.04
ATOM   1709  CA   LEU  221      30.498  23.666  28.697  1.00 13.22
ATOM   1710  C    LEU  221      31.309  24.178  29.887  1.00 10.73
ATOM   1711  O    LEU  221      31.846  25.267  29.857  1.00 12.98
ATOM   1712  CB   LEU  221      31.382  23.102  27.549  1.00 13.74
ATOM   1713  CG   LEU  221      32.580  22.257  28.045  1.00 18.64
ATOM   1714  CD1  LEU  221      32.149  20.868  28.496  1.00 17.38
ATOM   1715  CD2  LEU  221      33.571  22.109  26.911  1.00 26.97
ATOM   1716  N    GLU  222      31.316  23.446  30.963  1.00  9.31
ATOM   1717  CA   GLU  222      31.936  23.929  32.144  1.00  9.97
ATOM   1718  C    GLU  222      32.548  22.803  32.951  1.00 12.94
ATOM   1719  O    GLU  222      32.072  21.662  32.966  1.00 13.38
ATOM   1720  CB   GLU  222      30.836  24.762  32.896  1.00 12.14
ATOM   1721  CG   GLU  222      31.092  25.119  34.364  1.00 13.88
ATOM   1722  CD   GLU  222      29.895  25.891  34.934  1.00 13.57
ATOM   1723  OE1  GLU  222      29.128  26.477  34.240  1.00 19.47
ATOM   1724  OE2  GLU  222      29.752  25.789  36.207  1.00 18.51
ATOM   1725  N    PHE  223      33.687  23.123  33.542  1.00 15.86
ATOM   1726  CA   PHE  223      34.476  22.227  34.373  1.00  9.34
ATOM   1727  C    PHE  223      34.711  22.864  35.722  1.00 11.08
ATOM   1728  O    PHE  223      35.028  24.055  35.828  1.00 19.86
ATOM   1729  CB   PHE  223      35.847  21.919  33.684  1.00  8.30
ATOM   1730  CG   PHE  223      35.703  21.134  32.431  1.00 10.50
ATOM   1731  CD1  PHE  223      35.570  19.747  32.469  1.00 13.56
ATOM   1732  CD2  PHE  223      35.750  21.750  31.184  1.00 11.32
ATOM   1733  CE1  PHE  223      35.481  19.010  31.287  1.00 12.58
ATOM   1734  CE2  PHE  223      35.667  21.032  29.995  1.00 12.17
ATOM   1735  CZ   PHE  223      35.521  19.648  30.050  1.00 10.87
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FIG 5-27

ATOM	1736	N	VAL	224	34.542	22.081	36.765	1.00	9.28
ATOM	1737	CA	VAL	224	34.708	22.587	38.080	1.00	11.18
ATOM	1738	C	VAL	224	35.324	21.553	39.010	1.00	17.82
ATOM	1739	O	VAL	224	34.848	20.418	39.137	1.00	13.17
ATOM	1740	CB	VAL	224	33.370	23.078	38.662	1.00	16.61
ATOM	1741	CG1	VAL	224	33.622	23.736	40.022	1.00	13.90
ATOM	1742	CG2	VAL	224	32.674	24.048	37.697	1.00	13.85
ATOM	1743	N	THR	225	36.380	21.965	39.676	1.00	11.71
ATOM	1744	CA	THR	225	37.026	21.099	40.617	1.00	12.61
ATOM	1745	C	THR	225	37.366	21.798	41.927	1.00	14.76
ATOM	1746	O	THR	225	37.702	23.002	41.962	1.00	16.64
ATOM	1747	CB	THR	225	38.162	20.279	40.014	1.00	20.38
ATOM	1748	OG1	THR	225	39.288	20.337	40.822	1.00	30.44
ATOM	1749	CG2	THR	225	38.468	20.722	38.631	1.00	10.89
ATOM	1750	N	ALA	226	37.222	21.065	43.011	1.00	7.89
ATOM	1751	CA	ALA	226	37.478	21.595	44.352	1.00	11.63
ATOM	1752	C	ALA	226	38.969	21.558	44.677	1.00	16.61
ATOM	1753	O	ALA	226	39.687	20.699	44.199	1.00	15.60
ATOM	1754	CB	ALA	226	36.695	20.847	45.444	1.00	12.17
ATOM	1755	N	ALA	227	39.395	22.490	45.479	1.00	13.95
ATOM	1756	CA	ALA	227	40.789	22.550	45.871	1.00	19.64
ATOM	1757	C	ALA	227	40.987	23.299	47.170	1.00	26.33
ATOM	1758	O	ALA	227	40.042	23.715	47.840	1.00	25.39
ATOM	1759	CB	ALA	227	41.557	23.246	44.760	1.00	18.42
ATOM	1760	N	GLY	228	42.245	23.476	47.523	1.00	23.28
ATOM	1761	CA	GLY	228	42.616	24.292	48.658	1.00	21.61
ATOM	1762	C	GLY	228	42.805	23.562	49.939	1.00	32.93
ATOM	1763	O	GLY	228	42.948	24.201	51.009	1.00	32.53
ATOM	1764	N	ILE	229	42.803	22.231	49.842	1.00	33.59
ATOM	1765	CA	ILE	229	43.006	21.375	50.998	1.00	31.81
ATOM	1766	C	ILE	229	44.016	20.291	50.633	1.00	28.78
ATOM	1767	O	ILE	229	45.090	20.176	51.246	1.00	96.02
ATOM	1768	CB	ILE	229	41.691	20.772	51.519	1.00	35.70
ATOM	1769	CG1	ILE	229	40.890	21.807	52.325	1.00	30.66
ATOM	1770	CG2	ILE	229	41.990	19.549	52.392	1.00	33.37
ATOM	1771	CD1	ILE	229	39.386	21.715	52.092	1.00	38.74
ATOM	1772	O	HOH	301	27.530	12.735	38.010	1.00	15.09
ATOM	1773	O	HOH	302	23.919	34.589	37.331	1.00	10.29
ATOM	1774	O	HOH	303	27.229	34.816	35.487	1.00	11.12
ATOM	1775	O	HOH	304	29.914	18.943	44.692	1.00	16.10
ATOM	1776	O	HOH	305	30.956	21.886	49.900	1.00	21.47
ATOM	1777	O	HOH	306	20.072	31.196	43.592	1.00	16.85
ATOM	1778	O	HOH	307	26.660	48.630	33.797	1.00	24.67
ATOM	1779	O	HOH	308	22.329	33.239	41.399	1.00	14.11
ATOM	1780	O	HOH	309	22.465	48.025	32.810	1.00	18.51
ATOM	1781	O	HOH	310	31.012	39.126	29.118	1.00	16.01
ATOM	1782	O	HOH	311	33.067	35.809	33.010	1.00	19.92
ATOM	1783	O	HOH	312	31.130	37.076	30.841	1.00	12.58
ATOM	1784	O	HOH	313	40.304	30.058	38.616	1.00	56.07
ATOM	1785	O	HOH	314	34.166	26.379	57.222	1.00	22.58
ATOM	1786	O	HOH	315	36.215	35.320	43.598	1.00	22.30
ATOM	1787	O	HOH	316	33.866	29.786	34.671	1.00	12.21
ATOM	1865	O	HOH	317	42.341	20.166	43.534	1.00	26.67
ATOM	1788	O	HOH	318	10.270	28.684	30.403	1.00	43.65
ATOM	1789	O	HOH	319	28.448	16.822	30.655	1.00	25.44
ATOM	1790	O	HOH	320	30.612	20.922	37.231	1.00	21.57
ATOM	1791	O	HOH	321	11.639	37.421	26.801	1.00	34.12
ATOM	1792	O	HOH	322	27.030	37.308	36.869	1.00	13.10
ATOM	1793	O	HOH	323	33.119	14.524	43.070	1.00	30.93
ATOM	1794	O	HOH	324	37.973	14.036	53.352	1.00	35.39
ATOM	1795	O	HOH	325	32.015	49.100	37.028	1.00	55.37
ATOM	1796	O	HOH	326	11.959	12.020	43.429	1.00	29.06
ATOM	1797	O	HOH	327	36.760	29.941	31.666	1.00	22.03
ATOM	1864	O	HOH	328	15.305	26.513	15.694	1.00	35.62
ATOM	1798	O	HOH	329	33.005	46.924	26.994	1.00	22.07
ATOM	1363	O	HOH	330	23.501	36.134	22.715	1.00	46.30
ATOM	1799	O	HOH	331	33.609	31.296	26.261	1.00	23.65

FIG 5-28

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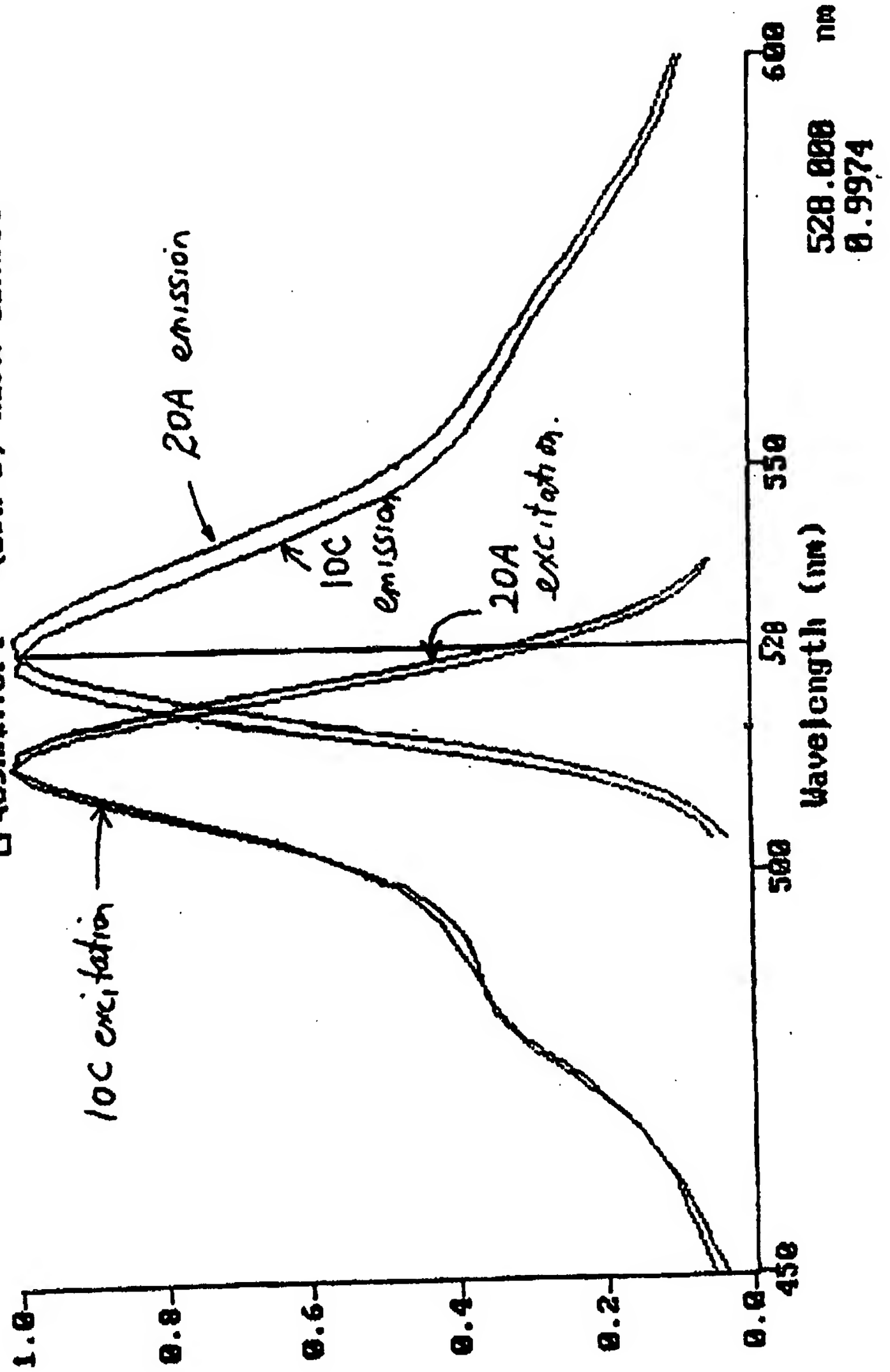
ATOM   1862  O   HOH   332      34.942  24.780  29.532  1.00 38.93
ATOM   1800  O   HOH   333      25.235  12.919  54.611  1.00 36.20
ATOM   1861  O   HOH   334      38.048  23.467  36.645  1.00 37.73
ATOM   1801  O   HOH   335      12.284  43.511  38.338  1.00 33.79
ATOM   1802  O   HOH   336       9.826  47.020  32.568  1.00 46.67
ATOM   1803  O   HOH   337       7.671  41.532  29.806  1.00 40.88
ATOM   1804  O   HOH   338      15.430  23.713  26.808  1.00 34.73
ATOM   1805  O   HOH   339      24.344  20.385  25.121  1.00 53.42
ATOM   1806  O   HOH   340      31.550  10.656  40.819  1.00 47.85
ATOM   1807  O   HOH   341      17.569  23.030  25.796  1.00 28.17
ATOM   1808  O   HOH   342      19.174  38.552  23.965  1.00 45.54
ATOM   1809  O   HOH   343      24.268  37.527  25.415  1.00 30.97
ATOM   1810  O   HOH   344      21.266  29.482  41.551  1.00 19.69
ATOM   1811  O   HOH   345      20.668  26.999  41.933  1.00 11.81
ATOM   1812  O   HOH   346      24.780  24.795  43.460  1.00 20.95
ATOM   1813  O   HOH   347      42.962  13.170  46.312  1.00 31.00
ATOM   1814  O   HOH   348      32.322  14.088  47.013  1.00 28.20
ATOM   1815  O   HOH   349      31.708  13.186  49.679  1.00 35.57
ATOM   1816  O   HOH   350      22.408  35.801  50.514  1.00 40.71
ATOM   1817  O   HOH   351      25.366  47.090  42.583  1.00 38.15
ATOM   1818  O   HOH   352      27.243  47.647  43.977  1.00 41.55
ATOM   1819  O   HOH   353      29.868  45.076  42.906  1.00 29.32
ATOM   1820  O   HOH   354      14.175  22.269  42.680  1.00 74.11
ATOM   1821  O   HOH   355      13.414  10.739  35.791  1.00 29.92
ATOM   1822  O   HOH   356      20.338   9.974  37.765  1.00 30.46
ATOM   1823  O   HOH   357      23.520  40.420  24.953  1.00 29.75
ATOM   1824  O   HOH   358      25.718  41.692  26.023  1.00 30.43
ATOM   1825  O   HOH   359      26.826  38.466  25.345  1.00 31.72
ATOM   1826  O   HOH   360      37.768  42.373  25.123  1.00 41.53
ATOM   1827  O   HOH   361      40.078  42.268  25.852  1.00 37.12
ATOM   1828  O   HOH   362      31.483  38.677  22.083  1.00 54.21
ATOM   1829  O   HOH   363      33.891  37.723  30.126  1.00 23.35
ATOM   1860  O   HOH   364      39.936  26.543  36.329  1.00 47.93
ATOM   1830  O   HOH   365      36.631  34.210  41.636  1.00 62.74
ATOM   1831  O   HOH   366      37.038  29.783  52.197  1.00 40.07
ATOM   1832  O   HOH   367      37.289  37.407  40.231  1.00 37.59
ATOM   1833  O   HOH   368      18.930  17.517  52.472  1.00 35.80
ATOM   1834  O   HOH   369      19.506  18.914  57.913  1.00 45.72
ATOM   1835  O   HOH   370      30.903  26.708  41.139  1.00 21.54
ATOM   1836  O   HOH   371      30.369  25.678  24.583  1.00 22.46
ATOM   1837  O   HOH   372      21.000  33.705  20.826  1.00 26.00
ATOM   1838  O   HOH   373      13.648  32.794  21.329  1.00 27.98
ATOM   1839  O   HOH   374      29.735  25.683  38.707  1.00 21.00
ATOM   1859  O   HOH   375      33.670  24.419  60.503  1.00 50.04
ATOM   1840  O   HOH   376      30.034  11.047  37.420  1.00 43.28
ATOM   1841  O   HOH   377       8.662  35.846  35.068  1.00 51.94
ATOM   1842  O   HOH   378      10.847  36.466  39.503  1.00 42.32
ATOM   1843  O   HOH   379      14.395  48.943  39.085  1.00 29.72
ATOM   1844  O   HOH   380      36.676  11.660  40.172  1.00 39.81
ATOM   1845  O   HOH   381      35.968   7.212  34.763  1.00 58.66
ATOM   1846  O   HOH   382      17.426  21.988  21.077  1.00 41.69
ATOM   1847  O   HOH   383      29.837  22.623  39.378  1.00 32.82
ATOM   1848  O   HOH   384      23.855  29.386  55.164  1.00 55.00
ATOM   1849  O   HOH   385      17.408  35.360  47.495  1.00 61.61
ATOM   1850  O   HOH   386      27.900  49.720  42.448  1.00 47.70
ATOM   1851  O   HOH   387      13.932  36.230  44.385  1.00 45.08
ATOM   1852  O   HOH   388      12.650  38.021  43.288  1.00 49.86
ATOM   1853  O   HOH   389      16.974  42.367  43.435  1.00 34.38
ATOM   1854  O   HOH   390      37.335  42.653  28.295  1.00 64.46
ATOM   1855  O   HOH   391      29.701  49.856  35.323  1.00 62.61
ATOM   1856  O   HOH   392      27.267  50.835  33.976  1.00 66.60
ATOM   1857  O   HOH   393      19.661  39.121  51.537  1.00 34.01
ATOM   1858  O   HOH   394      29.412  17.505  59.089  1.00 51.78
TER
END
```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10CND2N.SPT 10c non-mut, norm=1ex498
Q69KHN.SPT Q69K-8, norm=1ex498
Q69KDN.SPT Q69K-8, norm=1em544
20A emission
10C excitation
10C emission
20A excitation
600
528.000 nm
0.9974
500
528
550
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
450
Normalized Intensity (cps)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14593

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/04; C07K 14/00, 16/00; C12N 1/20, 15/00, 15/09, 15/63

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/252.3, 252.33, 325, 410, 320.1; 530/350, 387; 536/23.1, 23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	WO 96/23810 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 08 August 1996, abstract and claims.	46--53, 88, 92, 93, 94 ——— 1-41, 54-7
X — Y	HEIM et al. Improved green fluorescence. Nature. Vol. 373, 23 February 1995, pages 663-664, see Figure on page 664.	88 ——— 2, 3, 10-16, 18-26, 28-32, 34-37, 39-41

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 18 DECEMBER 1997	Date of mailing of the international search report 27 JAN 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer NASHAAT T. NASHED Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14593

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	HEIM et al. Wavelength mutations and posttranslational autoxidation of green fluorecent protein, Proc. Natl. Acad. Sci. USA. Vol. 91, December 1994, pages 12501-12504, see abstract.	46, 48, 50, 52, 54, 56, 94 ---------- 1-41, 47, 49, 51, 53, 55, 57
Y	PEROZZO et al. X-ray diffraction and time-resolved fluorescence analysis of Aequorea green fluorescent crystals. Journal of Biological Chemistry. 05 June 1988, Vol. 263, No. 16, pages 7713-7716.	1-41, 46-57, 86-94
X ----- Y	DELAGRAVE et al. Red-shifted excitation mutants of the green fluorescent protein. Bio/Technology. February 1995, Vol. 13, pages 151-153, see Table 1 on page 152.	46-57, 86-88, 91 ---------- 1-41
Y	EHRIG et al. Green-fluorescent protein mutants with altered fluorescence excitation spectra. FEBS Letters. 1995, Vol. 367, pages 163-166, abstract.	1-41, 46-57, 89, 90
Y	WANG et al. Implication for bcd mRNA localization from spatial distribution of exu protein in Drosophila oogenesis. Nature. 02 June 1994, Vol. 369, 400-403, see Figure 1.	32-41, 54-57
P, Y	ORMO et al. Crystal structure of the Aquorea victoria green fluorescent protein. Science. 06 September 1996, Vol. 273, pages 1392-1395, abstract.	1-41, 46-57, 86-94
P, Y	YANG et al. The molecular structure of green fluorescent protein. Nature Biotechnology. October 1996, Vol. 14, pages 1246-1251, abstract.	1-41, 46-57, 86-94
P, Y	PALM et al. The structural basis for spectral variations in green fluorescent protein. Nature Struct. Biol. May 1997, Vol. 4, Number 5, pages 361-365.	1-41, 46-57, 86-94
A	US 5,491,084 A (CHALFIE et al.) 13 February 1996, entire document.	1-41, 46-57, 86-94

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14593

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-41, 46-57, 86-94

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14593

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/252.3, 252.33, 325, 410, 320.1; 530/350, 387; 536/23.1, 23.4

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN: Medline, Caplus, Sciserch, Lifesci, Biosis, Embase, Wpids, Biotechds.
Search terms: aequorea and green fluorescent, T-203, Thr-203, T203, DNA, cDNA, sequence, s65t, t203h, s65t, t203y, s72a, f64l, s65g, 203y, s72a, s65g, v68l, t203y, l42x, v61x, t62x, v68x, q69x,n121x,y145x, v150x, f165x, i167x, q183x

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-41, drawn to DNA coding for mutant fluorescent green protein having mutation at Thr-203, the fluorescent protein, antibody labeled with the fluorescent protein and the DNA coding for a fusion protein consisting of an antibody and the mutant fluorescent protein.

Group II, claims 42-45 and 58-61, drawn to a DNA probe labled with mutants fluorescent.

Group III, claims 46-57 and 86-94, drawn to drawn to DNA coding for mutants fluorescent green protein having mutation at an amino acid residue other than Thr-203, the fluorescent protein, antibody labeled with the fluorescent protein and the DNA coding for a fusion protein consisting of an antibody and the mutant fluorescent protein.

Group IV, claims 62-64 and 68-70, drawn to a method for engineering fluorescent protein.

Group V, claims 65-67, drawn to method of producing fluorescent resonance energy transfer.

Group VI, claims 71-74, drawn to a fluorescent protein crystal having the amino acid sequence SEQ ID NO: 2.

Group VII, claims 75-82, drawn to a computation method for the design of fluorescent protein.

Group VIII, claims 83-85, drawn to a storage device containing the atomic coordinate.

Group IX, claims 95-100, drawn to a method of identifying test chemicals.

The inventions listed as Groups I-IX do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Each of the above group has a special technical feature defined by the first claim in the Group. The following are the special technical feature for each Groups: (a) Group I is the nucleic acid coding for fluorescent protein having at least the mutation at Thr-203, (b) Group II is a fluorescent DNA probe labeled with a mutant fluorecent protein, (c)Group III is the nucleic acid sequence coding for mutant fluorescent protein having mutation at residues other than T-203, (d) Group IV is a method for the engineering of mutant fluorescent proteins, (e) Group V is a method for producing fluorescent resonance energy transfer, (f) Group VI is the protein crystal of the wild-type protein, (g) Group VII is the computation method to design mutants fluorescent protein with different fluorescent characteristics, (h) Group VIII is a storage device for data, and (i) Group IX is a method of identifying test chemicals.

Group I encompasses the nucleic acid coding for the mutant fluorescent protein, expression vector, recombinant host cell, the mutant proteins and a use for the DNA in making the fusion protein consisting of antibody and the fluorescent protein. Group II represent a second use for the mutant protein of Group I. Also, the special technical feature of Group I is different from that of Group III because the DNA of each Group codes for different sets of mutants that do not share common feature. The special technical feature for this Group I is distinct from those of Groups IV-IX.

The special technical feature of Group II, the fluorescent DNA probe is clearly different from those of Groups III-IX. The DNA probe of Group II represent a second use of the fluorescent protein of Group II.

INTERNATIONAL SEARCH REPORT

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The method of engineering fluorescent protein of Group IV is different from that of producing fluorescent resonance energy transfer of Group V because the resulting fluorescence is different in each case and vary in its characteristics. Similarly, the special technical features of each of Groups IV and V are different from those of the crystal of Group VI, the computation method of Group VII, the storage device of Group VIII, and the method of identifying chemicals of Group IX. Finally, the crystal of Group VI, the computation method of Group VII, the storage device of Group VIII, and the method of identifying chemicals of Group IX are clearly unrelated to each other and there is no special technical feature that connects them together.